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PROCEEDINGS OF
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EFFECT OF ADRENALECTOMY ON THE TESTIS OF CADMIUM CHLORIDE TREATED RATS

by Amiya B. Kar, J. N. Karkun *and* R. P. Das,* *Central Drug Research Institute, Lucknow*

Abstract

Adrenalectomy caused an increased proliferation of fibroblasts in the testis of CdCl₂ treated rats but the rate of formation of Leydig cells was not altered.

Following adrenalectomy there was mass deposition of fibrous tissue in the interstitial spaces and an increase in alkaline phosphatase activity in the cellular debris contained in the necrosed tubules of CdCl₂ injected rats.

Adrenalectomy was partially conducive to the morphogenetic recovery of the interstitium from the adverse effects of CdCl₂. The resumption of endocrine activities, on the other hand, was not influenced by the operative procedure.

Besides causing an increase in alkaline phosphatase activity in the cellular debris, adrenalectomy had no effect on the necrosed seminiferous tubules.

Introduction

Parizek (1957) reported that Cd salts caused acute destruction of the gametogenic and interstitial elements of the testis in rats. Mild necrotic changes appeared within first 6 hours after the injection of CdCl₂, and 48 hours later the seminiferous epithelium was totally destroyed. In the interstitial tissue there were focal haemorrhages, vascular thromboses and a modicum of inflammatory reaction. However, after 10 days the entire organ was replaced by masses of eosinophilc debris containing scattered residue of basophilic chromatin material. It was interesting that during the subsequent days, while the central portion of the testis remained necrotic, there was proliferation of fibroblasts and blood vessels in peripheral regions underneath the tunica albuginea. Islets of Leydig cells were also seen in these areas and this was followed by a gradual return of the endocrine activities of the testis. Initially, the necrotic changes induced by Cd evoked castration phenomena but the atrophied accessory sexual organs retained their intrinsic ability to react to androgenic stimulation. Kar *et al.* (1959) noted that CdCl₂ compromised the stimulatory action of testosterone propionate on the accessory genital organs of castrated rats : a direct action of Cd on these organs was also recorded in such animals.

The present investigation was designed to study the effect of adrenalectomy on the rate of regeneration of interstitial elements of the testis in CdCl₂ treated rats. As the glucocorticoids were known to have an inhibitory effect on the proliferation of fibroblasts (Noble, 1955), it was envisaged that the removal of adrenals at an appropriate time might provide a fillip to the regeneration of the interstitium by *mass* proliferation of these elements. The latter process in its turn might be expected to lead to the formation of Leydig cells in commensurately larger numbers, as it was satisfactorily established that the fibroblasts were the precursors of Leydig cells (Charney *et al.*, 1952 ; Sniffen, 1952 ; Fawcett and Burgos, 1956).

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* I. C. M. R. Research Fellow.

EXPERIMENTAL PROCEDURE

Adult albino rats of the Institute Colony were used in this investigation. Details of grouping of the animals and their body weights are indicated in Table I. All the animals were maintained under uniform laboratory conditions throughout the experimental period.

$CdCl_2$ was administered by the subcutaneous route (0.04 m. mol. $CdCl_2$/kg. body weight at the interscapular region, single injection). Adrenalectomy was performed 8 days after $CdCl_2$ treatment in order to ensure the removal of circulating corticoids a little before the commencement of regenerative processes in the interstitium. It may be recalled that the proliferation of interstitial elements started 10 days after the administration of $CdCl_2$ (Parizek, 1957). A group of normal animals was also adrenalectomized and all the operated animals together with the normal controls, were maintained on 0.9 per cent physiological saline solution.

The animals (including normal and untreated adrenalectomized) were sacrificed on the 30th day after the administration of $CdCl_2$. The testis, seminal vesicles (SV) and the ventral prostate (VP) were carefully dissected out and weighed to the nearest mg. For histological studies the testis were fixed in alcoholic Bouin's fluid and serial paraffin sections were stained with Ehrlich's hematoxylin followed by eosin. Sections from the same series were stained with Mallory's trichrome stain for the demonstration of fibrous connective tissue. Alkaline phosphatase activity was studied in paraffin sections of the testis fixed in a chilled absolute ethanol-acetic acid mixture (Wolman and Behar, 1952), by the technique of Gomori (1941) as laid down by Glick (1949). The sections were incubated in the substrate for 48 hours and were mounted without counterstaining. Total cholesterol content of the testis was estimated colorimetrically by the modification (Karkun and Das, *unpublished*) of a method by Zlatkis *et al.* (1953).

RESULTS

Testis. It will be evident from Table I that adrenalectomy caused a significant decrease in absolute weight of the testis (P .001) but the relative weight remained virtually unaltered. It was to be noted that the body weight gain was considerably impeded in the operated animals (as compared to the controls) and this was probably responsible for such apparent loss in absolute weight of the testis. $CdCl_2$ treatment, on the other hand, evoked a significant reduction in absolute and relative weights of the organ (P .001). However, adrenalectomy in $CdCl_2$ injected animals tended to elevate the testis weight (absolute and relative) so that the difference from that of the intact $CdCl_2$ treated group was statistically significant (P .05). The relative testis weight of the $CdCl_2$ injected adrenalectomized animals did not reveal a statistically significant increase as compared to the group (intact) treated with $CdCl_2$. Nevertheless, it was interesting that the testis weight (absolute and relative) of the operated plus $CdCl_2$ injected group was significantly lower than that of the normal or adrenalectomized controls (P .001).

Histologically, the testis of the normal controls showed full spermatogenesis ; the tubules exhibited vigorous activity with successive stages of transformation of the seminiferous epithelium into mature spermatozoa. The latter were present in large numbers and their disposition was typical (Fig. 1). There were numerous Leydig cells in the interstitium and the vascularity of the entire organ was normal.

In Mallory preparations the thin basement membrane of the tubules stained blue (Fig. 1a). The other elements which showed similar tinctorial reaction were the tunica albuginea and the serosa of the interstitial blood vessels.

Adrenalectomy was without any effect on spermatogenesis (Fig. 2). In general, the Leydig cells appeared normal with no obvious symptoms of atrophy. However, in some locations atrophic Leydig cells with pycnotic nuclei were encountered.

TABLE 1

Testis weight, testis cholesterol and weight of the accessories of adrenalectomized CdCl₂ treated rats.

Treatment	Mean testis weight with S.E.		Mean testis cholesterol with S.E. (Mg./gm. testis)	Mean seminal vesicle weight with S.E.		Mean ventral prostate weight with S.E.		Mean body weight (G.M.) with S.E.	
	Absolute (Mg.)	Relative (Mg./100 gm. body weight)		Absolute (Mg.)	Relative (Mg./100 gm. body weight)	Absolute (Mg.)	Relative (Mg./100 gm. body weight)	Initial	Final
Normal Controls	1022.9±30.67 (6)*	736.4±25.59 (6)	8.38±0.24 (6)	64.52±9.52 (6)	45.52±5.52 (6)	98.42±8.17 (6)	70.85±6.54 (6)	115.3±3.13 (6)	139.3±4.43 (6)
Adrenal-ectomized	830.9±50.56 (7)	748.5±24.61 (7)	8.45±0.14 (7)	29.34±4.04 (7)	27.16±2.81 (7)	54.53±9.95 (7)	48.63±8.63 (7)	106.4±3.38 (8)	109.5±4.94 (8)
CdCl₂	146.4±13.77 (7)	129.04±15.86 (7)	4.12±0.19 (7)	7.81±0.65 (8)	6.60±0.45 (8)	14.00±0.67 (8)	12.28±0.97 (8)	95.4±2.41 (8)	107.4±2.35 (8)
Adrenal-ectomized + CdCl₂	210.7±26.72 (6)	148.9±17.05 (6)	4.90±0.37 (6)	12.10±1.95 (6)	11.60±3.42 (6)	16.07±1.79 (6)	11.40±1.05 (6)	89.8±6.35 (6)	149.7±7.55 (6)

*Figure in parenthesis indicates the number of animals.

Nevertheless, considering the preponderance of normal cells such atrophic ones formed only a fraction of the total Leydig cell population.

In sections stained with Mallory's the histological features of the testis of adrenalectomized animals were similar to those of the normal controls. The thin basement membrane of the tubules, the serosa of the interstitial blood vessels and the tunica albuginea were the only elements which stained blue (Fig. 2a).

$CdCl_2$ treatment caused complete destruction of the seminiferous tubules. The epithelial elements were totally necrosed and the tubular lumen was filled with an eosinophilic debris containing scattered residue of basophilic chromatin material (Fig. 3). Proliferation of fibroblast was clearly seen under the tunica albuginea and the deposition of peritubular fibrous tissue was also not uncommon. In some necrosed tubules the fibroblasts even invaded the eosinophilic debris. There was proliferation of blood vessels under the tunica albuginea and clumps of Leydig cells were also seen in this area. In contrast to the more central portions of the testis, the peripheral areas contained more fibroblasts and greater amounts of peritubular fibrous tissue (Fig. 3). Such histological difference between the two areas was more prominent in Mallory preparations (Fig. 3a). It was also noteworthy that the tunica albuginea was considerably thickened and stained a deep blue with Mallory's.

In $CdCl_2$ treated adrenalectomized animals the necrosis of the tubules persisted throughout the testis. However, in most of the animals there were more fibroblasts and greater amounts of fibrous tissue than in the unoperated group injected with $CdCl_2$. This could be gauged from the fact that the fibroblasts invaded even the central portions of the testis and the amount of fibrous tissue was almost equal throughout the organ (Fig. 4). It may be recalled that in the intact $CdCl_2$ treated group the fibroblastic proliferation and deposition of fibrous tissue were virtually confined to the peripheral areas underneath the tunica albuginea. It was also interesting that in the latter group the disposition of fibrous tissue was predominently peritubular whereas, in the adrenalectomized animals (given $CdCl_2$) this tissue was seen in varying amounts thoughout the interstitial space with characteristic peritubular concentration, particularly underneath the tunica albuginea (Fig. 4). Leydig cells were present mostly in the peripheral areas but their number did not increase after adrenalectomy. Vascular proliferation was also seen in these areas. Mallory preparations demonstrated this equal distribution of fibroblasts and fibrous tissue in the two regions (central and peripheral) of the testis even more clearly (Fig. 4a). Further, the characteristic blue staining reaction confirmed the presence of fibrous tissue *throughout* the interstitial space.

In agreement with the findings of Dempsey *et al.* (1949) and Kar *et al.* (1950) it was seen that in the control animals the basement membrane of the tubules and the spermatogonia showed intense phosphatase activity (Fig. 5). The other elements of the seminiferous epithelium contained only moderate amounts of the enzyme. The Leydig cells and the endothelium of the interstitial vessels were strongly reactive.

Adrenalectomy caused an overall reduction in alkaline phosphatase activity in the testis. The seminiferous epithelium was virtually devoid of enzyme activity except in the nucleus of the spermatogonia (Fig. 6). The Leydig cells and the endothelium of the interstitial vessels contained very little alkaline phosphatase activity.

Testicular phosphatase activity was markedly inhibited after $CdCl_2$ treatment. However, some of the necrosed tubules underneath the tunica albuginea retained considerable amounts of the enzyme ; the cellular debris in these tubules was the specific reactive material (Fig. 7). The centrally located tubules were negative for alkaline phosphatase activity except a few which showed only a faint reaction in the cellular debris. Other elements of the testis were invariably devoid of enzyme activity.

The pattern of distribution of alkaline phosphatase in the testis of $CdCl_2$ treated adrenalectomized animals was the same as in the intact group injected with $CdCl_2$. Thus the enzyme activity was confined to the debris of the necrosed tubules albeit in more intense manner than in the previous group (Fig. 8). Further, phosphatase activity was seen in greater number of tubules both in the central and peripheral areas. Other elements of the testis, however, continued to give negative reactions for enzyme activity.

Adrenalectomy did not evoke any change in total cholesterol content of the testis (Table I). However, $CdCl_2$ treatment (intact or adrenalectomized animals) caused a significant reduction in testicular cholesterol concentration as compared to either normal or adrenalectomized controls (P $<$ .001). It was to be noted that there was no significant difference between the two $CdCl_2$ treated groups as regards any aberration in cholesterol content of the testis.

Seminal vesicles. Adrenalectomy caused a significant reduction in absolute and relative weights of the SV (P $<$ .02). $CdCl_2$ treatment (adrenalectomized or intact animals) was associated with a more drastic reduction in weight (absolute and relative) of this organ as compared to either normal or adrenalectomized controls (P $<$ .001). The SV weight (absolute and relative) of the two $CdCl_2$ treated groups did not differ significantly (Table I)

Ventral prostate. The absolute weight of the VP was significantly lowered after adrenalectomy (P $<$ .01); but the relative weight of the organ did not reveal a statistically significant difference inspite of the fact that it was appreciably less than that of the normal controls. $CdCl_2$ treatment (normal and adrenalectomized animals) was responsible for a significant decrease in VP weight (absolute and relative) as compared to either normal or adrenalectomized controls (P $<$ .001). The two $CdCl_2$ treated groups did not differ significantly with respect to their VP weights (Table I).

DISCUSSION

The data presented in this report indicated that adrenalectomy did influence certain morphogenetic events in the interstitium during its recovery from the adverse effects of $CdCl_2$. On the other hand, the seminiferous tubules were irreversibly destroyed and adrenalectomy failed to exert any recuperative effect on the defunct epithelial remnants.

Parizek (1957) noted that during progressive necrosis of the testis after administration of $CdCl_2$, a stage was reached when the gametogenic and the interstitial portions of the testis appeared to be equally affected. But subsequently while the spermatogenic elements continued to persist merely as a morass of dead tissue, focal regenerative changes commenced in the interstitium. There was no doubt that these changes heralded both morphogenetic and functional recovery of the interstitium; as clumps of active Leydig cells, presumably formed from the proliferated fibroblasts were consistently encountered. It was precisely this process of fibroblastic proliferation which lent itself to experimental modification for a prompt and more complete resumption of endocrine activities of the interstitium. However, the reason why such modification was attempted specifically through the adrenocortical pathway was already indicated (*vide supra*).

Notwithstanding such provocative syllogism, the removal of adrenals did not prove conducive to a more complete functional recovery of the interstitium. There was no doubt that the fibroblasts were proliferated in larger numbers than in the unoperated $CdCl_2$ treated animals but the Leydig cell population failed to show the expected increase, at least during the experimental period employed in this study. Further, the weight of the accessories in the two $CdCl_2$ treated groups (intact and adrenalectomized) was virtually similar which indicated that adrenalectomy did not improve the endocrine status of the regenerating interstitium

through an enhanced rate of formation of Leydig cells from their precursors. The similar cholesterol concentration of the testis in the two $CdCl_2$ treated groups also pointed towards such a conclusion.

Changes in alkaline phosphatase activity after $CdCl_2$ treatment merited a comment. It was noteworthy that the enzyme activity was markedly reduced in the testis after adrenalectomy. However, the administration of $CdCl_2$ was also associated with a similar overall decrease in phosphatase activity although the dead cellular debris in some necrosed tubules gave positive reactions for the enzyme. Curiously, adrenalectomy accelerated phosphatase activity in the cellular debris of such tubules. Whether the positive histochemical reaction in the cellular debris of defunct tubules was indicative of a true mobilization of the enzyme could not be determined from the present data.

ACKNOWLEDGEMENTS

REFERENCES

Charney, C. W., Conston, A. S. and Meranze, D. (1952). Testicular developmental histology. *Ann. N. Y. Acad. Sci.*, **55**, 597–608.
Dempsey, E. W., Greep, R. O. and Deane, H. W. (1949). Changes in the distribution and concentration of alkaline phosphatase in the tissues of the rat after hypophysectomy or gonadectomy and after replacement therapy. *Endocrinol.*, **44**, 88–103.
Fawcett, D. W. and Burgos, M. H. (1956). Observations on the cytomorphosis of the germinal and interstitial cells of the human testis. *Ciba Colloq. on Ageing*, **2**, 86.
Glick, D. (1949). Techniques of Histo- and Cytochemistry Interscience Publ., N.Y.
Gomori, G. (1941). The distribution of alkaline phosphatase in various cells and tissues. *J. Cell. Comp. Physiol.*, **17**, 71–83.
Kar, A. B., Banerjee, S. and Ghosh, A. (1950). The distribution of alkaline phosphatase in different tissues of alloxan diabetic rats. *Anat. Anz.*, Jenn, **98**, 336–343.
Kar, A. B., Karkun, J. N. and Das, R. P. (1959). Effect of cadmium chloride on the response of the genital organs of gonadectomized male and female rats to homologous sex hormones. *Ind. J. Med. Res.*, **47**, 24–28.
Karkun, J. N. and Das, R. P. (1958). *Unpublished data*.
Noble, R. L. (1955). The Hormones, **3**, Acad. Press, Inc., N.Y.
Parizek, J. (1957). The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. *J. Endocrinol.*, **15**, 56–63.
Sniffen, R. (1952). Histology of the normal and abnormal testis at puberty. *Ann. N.Y. Acad. Sci.*, **55**, 609–618.
Wolman, M. and Behar, A. (1952). A method of fixation for enzyme cytochemistry and cytology. *Expt. Cell. Res.*, **3**, 619–621.
Zlatkis, A., Zak, B. and Boyle, A. J. (1953). A new method for direct determination of serum cholesterol. *J. Lab. Clin. Med.*, **41**, 486–492.

EXPLANATION OF PLATE I

(All figures are photomicrographs. Figures 1, 1a, 2, 2a, 5 and 6 are magnified × 130)

Fig. 1. Testis of a normal rat. H and E.
Fig. 1a. Testis of a normal rat. Mallory's trichrome stain. The thin basement membrane of the tubules stains blue.
Fig. 2. Testis of an adrenalectomized rat. H and E. Full spermatogenesis as in the controls. Compare with fig. 1.
Fig. 2a. Testis of an adrenalectomized rat. Mallory's trichrome stain. Features similar as in fig. 1a.
Fig. 3. Testis of a $CdCl_2$ treated rat. H and E. The tubules are totally necrosed and contain a dead cellular debris. Note proliferation of fibroblasts (small black dots) under the tunica albuginea and peritubular deposition of fibrous tissue. The fibroblasts and fibrous tissue are more at the periphery (upper left of the fig.) than in the central portions of the testis (lower left and lower right of the fig.).
Fig. 3a. Testis of a $CdCl_2$ treated rat. Mallory's trichrome stain. Note the distribution of fibroblasts and fibrous tissue. The tunica albuginea is greatly thickened.
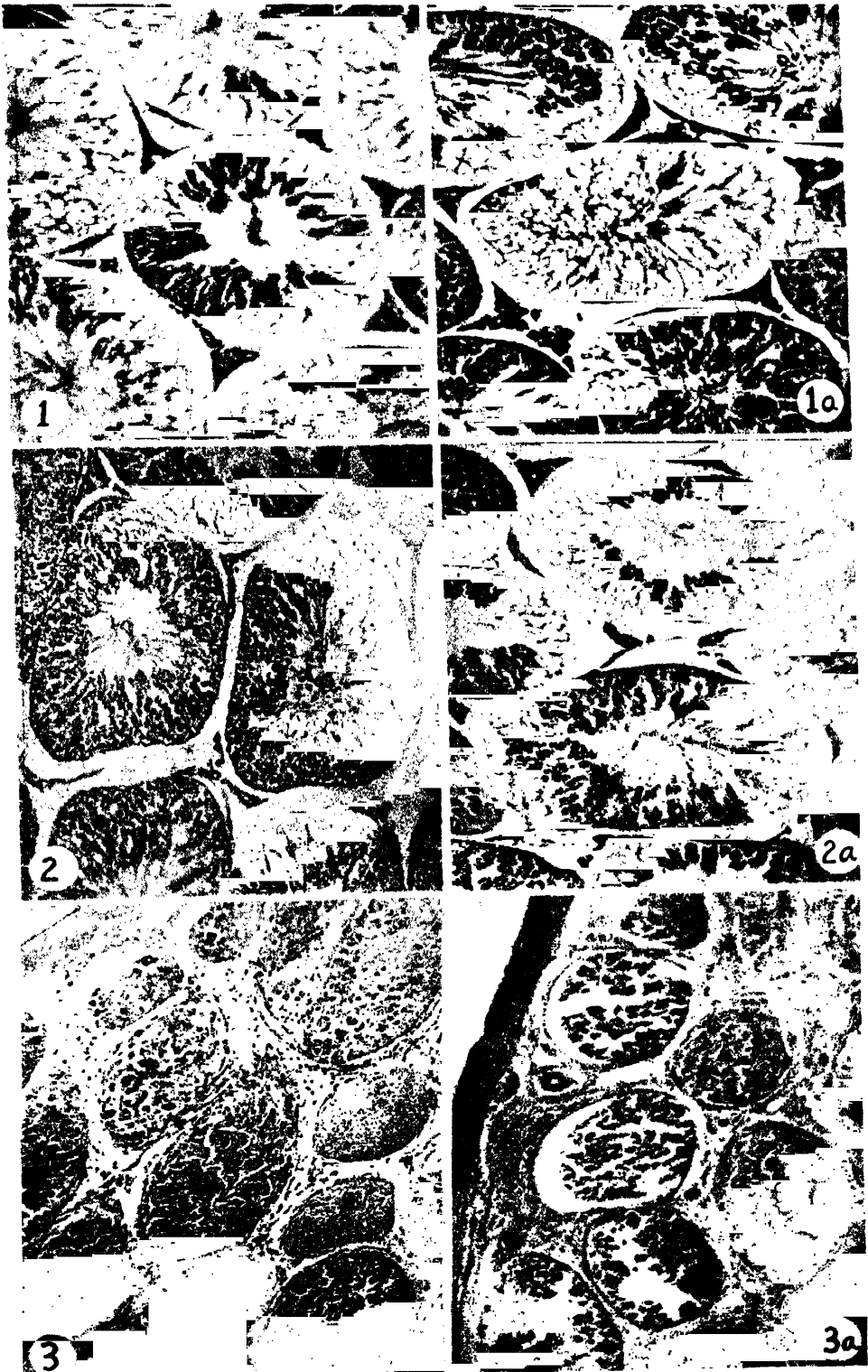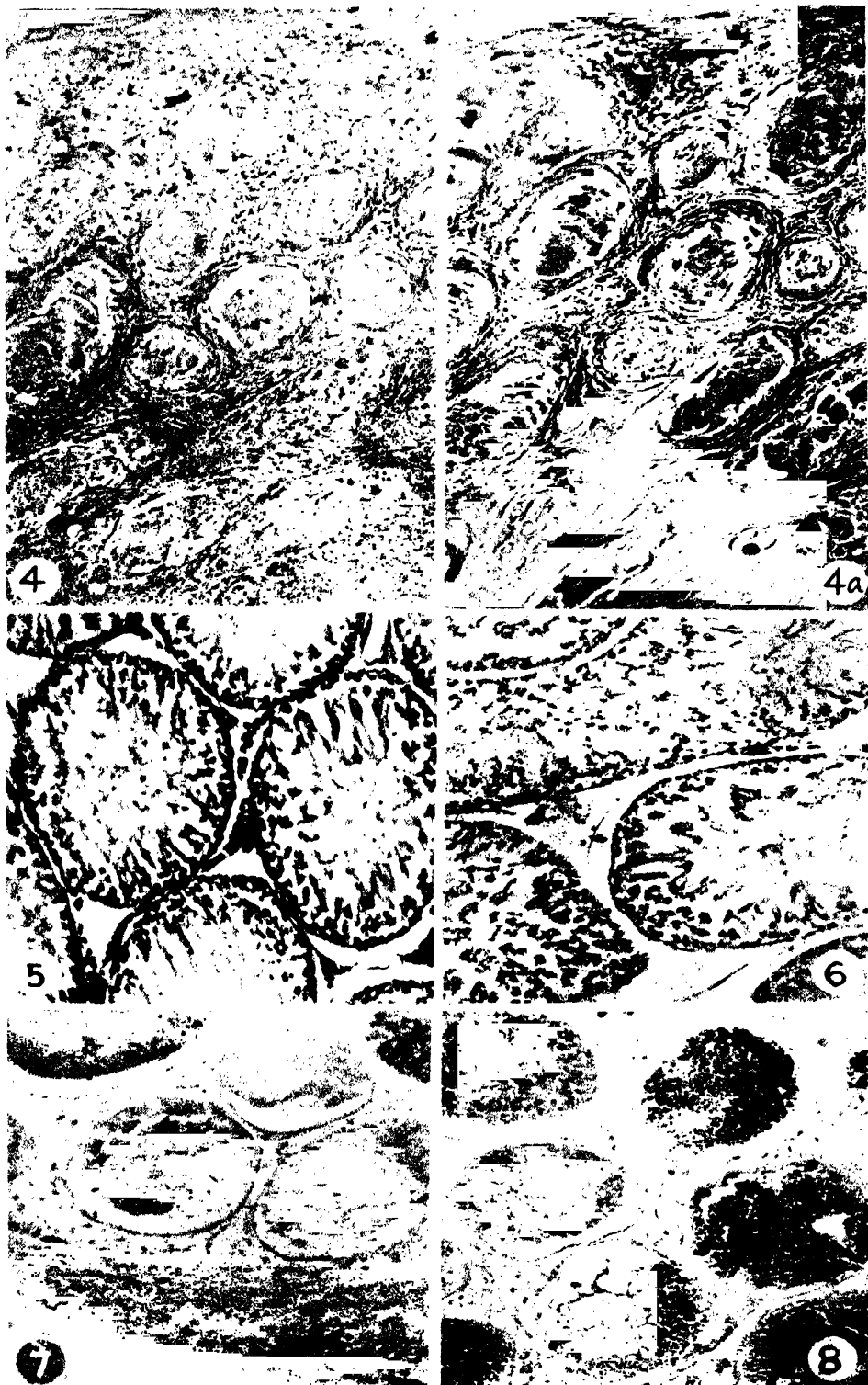
Fig. 4.   Testis of a CdCl$_2$ treated adrenalectomized rat. H and E. Note mass proliferation of fibroblasts (black dots) and deposition of fibrous tissue throughout the interstitial space with characteristic peritubular concentration. Compare with fig. 3.

Fig. 4a.   Testis of a CdCl$_2$ treated adrenalectomized rat. Mallory's trichrome stain. Note the deposition of fibrous tissue. Compare with fig. 3a.

Fig. 5.   Testis of a normal rat. Gomori technique. Note phosphatase activity in the basement membrane and spermatogonia. The Leydig cells are also highly reactive.

Fig. 6.   Testis of an adrenalectomized rat. Note overall reduction of enzyme activity.

Fig. 7.   Testis of a CdCl$_2$ treated rat. Gomori technique. The cellular debris in some tubules shows phosphatase activity.

Fig. 8.   Testis of a CdCl$_2$ treated adrenalectomized rat. Gomori technique. Note intense phosphatase activity in the cellular debris of the tubules. Compare with fig. 7.

ON THE ANTIBIOTIC PROPERTIES OF SOME CONSTITUENTS OF MESUA FERREA LINN.

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ABSTRACT

The antibiotic activity of mesuol, mesuone, and kernel and shell oils of *Mesua ferrea* seeds have been studied. Of these, mesuol and mesuone have been found to be fairly antibacterial. Mesuol is about 1 per cent as active as penicillin against *S. aureus*. None of the constituents have been found to be active against any of the fungi tested.

Mesua ferrea Linn. is a tree of tropical Asia and belongs to the family *Gutti ferae*. Various parts of the plant are used medicinally in India, Pakistan, Indochina and Malaya. The flowers are said to be astringent, stomachic, expectorant and useful in bleeding piles, the flower buds are used in dysentery. The unripe fruits are aromatic and sudorific. The bark is said to have similar properties. Leaves and flowers are used in the treatment of snake-bite and scorpion-sting (Chopra, Nayar and Chopra, 1956). The oil is used in soapmaking, and medicinally as an embrocation in rheumatism and in the treatment of itch (Kirtikar and Basu, 1935).

The oil of the seed-kernel has been investigated by Boorsma (1904), Hooper (1908), Grimme (1910), Dhingra and Hilditch (1931), and Chatterjee and Gupta (1937). The presence of an essential oil and two bitter substances has been reported in the flowers (Boorsma, 1904).

The seed was found to contain a pale yellow lactone, $C_{23}H_{22}O_5$, called mesuol, to the extent of 1 per cent (Dutt, Deb and Bose, 1940). Recent investigations have shown that another constituent, called mesuone, $C_{29}H_{42}O_4$, m.p. 136°, is also present in the seed kernel to the extent of 0.01 per cent. The structures of these compounds have not been definitely established although from the data at our disposal there is little doubt that mesuol is a 4-phenylcoumarin of a complex nature.

The shell oil has also been investigated by one of us (D.P.C.) and has been found to contain a phenolic fraction (unpublished data).

The antibacterial and antifungal properties of some natural coumarins observed by different investigators have recently been summarised by Bose (1958). Of the seventeen natural coumarins tested against the fungi, *Curvularia lunata* and *Aspergillus niger* psoralene, seselin, luvangetin and xanthyletin have been found to have pronounced action against *C. lunata* (Chakraborty, Das Gupta and Bose, 1957). Calophyllolide, a 4-phenylcoumarin derivative isolated from *Calophyllum inophyllum* Linn. (Fam. *Guttiferae*), has been found to have tuberculostatic activity (Ormancey-Potier, Buzas and Lederer, 1951). Novobiocin (Kaczka *et al.*, 1956 and Frost *et al.*, 1955-56), a derivative of 3-amino-4 : 7-dihydroxy -8-methyl coumarin has been reported to be highly antibacterial. It is almost as active as penicillin against *Staphylococcus sp.* and is also effective against bacteria resistant to other antibiotics.

In course of a study of biochemical behaviour of natural coumarins, it appeared to us of interest to study the action of some constituents of *M. ferrea* on a few test

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organisms of both fungal and bacterial origin. The results are presented here. The micro-organisms selected were *Micrococcus pyogens* var. *aureus* (*Staphylococcus aureus*), *Bacillus friedlanderi*, *Escherichia coli*, *Eberthella typhosa*, *Vibrio cholerae*, *Mycobacterium phlei*, *Curvularia lunata*, *Aspergillus niger*, *Helminthosporium sativum*, *Alternaria solani*, and on each of these the effects of mesuol, mesuone, and the kernel and shell oils of *M. ferrea* seeds were studied. The first two substances were used in concentrations which varied from 3 : 1000 to 3 : 100000. The oils were used without dilution.

The assay was carried out by the agar-cup method. None of the four constituents of *M. ferrea* was found to have any action on the test fungi. Consequently no data relating to these fungi have been presented.

The kernel oil showed a slight activity against *S. aureus*, but the shell oil showed no activity against any of the bacteria selected for test. Mesuol and mesuone had some activity against the bacteria. The activities of mesuol and mesuone were also compared with that of "Penicillin G" (Glaxo Laboratories) of potency 1650 units per mg. against *S. aureus* and with that of dihydrostreptomycin sulphate (American Cyanamid Co.) against *M. phlei*. Assay was also carried out in presence of normal horse serum. The data have been presented in Table I.

EXPERIMENTAL

Assay of antibacterial activity:—This was carried out by the agar-cup method. The test bacteria *S. aureus*, *E. coli*, *E. typhosa*, *V. cholerae*, *B. friedlanderi*, *M. phlei* were grown in nutrient broth, incubated at 37°C for 2 hours. Loopfuls of these were transferred to nutrient broth tube again and incubated for 2 hours. The broth (0.1 c.c.) culture was then diluted 100 times with sterile distilled water and this bacterial suspension was finally used as inoculum. One c.c. of this inoculum was thoroughly mixed with 25 c.c. of nutrient agar medium, previously melted and cooled to 45-46°C. The inoculated medium was poured into sterile Petri dishes (diameter 9 cm.).

Assay of antifungal properties:—This was also done by the agar-cup method. Conidial suspensions of the test organisms *A. niger*, *C. lunata*, *A. solani* and *H. sativum* were used as inocula. A loopful of conidia from a 8-day slant culture, grown on Czapek-Dox agar (CDA) medium was suspended in 10 c.c. sterile water. One c.c. of the spore suspension was added to 25 c.c. CDA medium previously melted and brought down to 45-46°C. The inoculated medium was poured into sterile Petri dishes as described above.

The agar plates solidified after sometime, and cups were cut out with sterile cork borer (bore diameter 8 mm.) and solutions of materials to be tested were put into the cups by means of sterile pipettes. The incubation temperatures for the bacterial and fungal plates were 37°C and 25°C respectively. The inhibition zones were measured after 24 and 48 hrs. in the case of bacterial and fungal test organisms respectively.

As mesuol and mesuone are insoluble in water, they were dissolved in propylene glycol. Along with the experimental assay materials, a control was always run for the solvent, propylene glycol, which showed a negligible inhibition zone. Solutions were prepared with 15 to 17 mg. of the substances and the volume was so made up that the concentrations reached 3 : 1000. This solution was used as such and was also subsequently diluted in ratios of 1 : 10, 1 : 100 and 1 : 1000 with propylene glycol for the purpose of further tests. Aqueous solutions of penicillin G and of dihydrostreptomycin sulphate were prepared by dissolving 15 mg. of the material in 5 c.c. sterile water. These were further diluted in ratios of 1 : 10 ; 1 : 100 ; 1 : 1000 and 1 : 10000 with water for further tests.

The shell oil as well as the kernel oil was used as such. Each experiment was repeated 4 to 5 times and the mean values were taken (Table I). The variation

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TABLE 1

Antibiotic Activities of Mesuol and Mesuone

Figures represent inhibition zones in mm.

Name of micro-organism	Concentration of mesuol			Concentration of mesuone			Concentration of Penicillin G			Concentration of dihydrostreptomycin sulphate		
	3 in 10^3	3 in 10^4	3 in 10^5	3 in 10^3	3 in 10^4	3 in 10^5	3 in 10^5	3 in 10^6	3 in 10^7	3 in 10^5	3 in 10^6	3 in 10^7
S. aureus	30(12)*	12(—)	12(—)	25(12)	18(—)	10(—)	40	30	20			
E. coli	11	-	-	14								
F. typhosa	12	-	-	13								
V. cholerae	12	-	-	13								
B. friedlanderi	11	-	-	14								
M. phlei	22(12)*	12(—)	-	18(12)	10(—)					15	15	12

*Figures in parentheses indicate inhibition zones in mm. in presence of 10 per cent normal horse serum.

in the diameter of the inhibition zones for each compound of the same concentration was within 1.5 mm. in the different experiments.

RESULTS AND DISCUSSION

It appears that of all the substances studied mesuol and mesuone are the most active against *S. aureus*. Mesuol is more active than mesuone against *M. phlei*. None of the constituents have been found to be active against any of the fungi tested.

It appears from the table that the minimum concentration at which mesuol and mesuone inhibit the growth of *S. aureus* is of the order of 30 micrograms per c.c. while that for penicillin is of the order of 0.30 microgram per c.c. From this it may be said that mesuol is about 1 per cent as active as penicillin against *S. aureus*. It may be mentioned here that allicin, a plant antibiotic, isolated from *Allium cepa*, (Cavallito and Bailey, 1944) when tested against *S. aureus* also showed an antibacterial activity of 1 per cent as compared to penicillin. The antibacterial activity of mesuol and mesuone against *S. aureus* is thus of the same order as that of allicin. The remarkable decrease of the *in vitro* activity of mesuol and mesuone in presence of serum suggests that *in vivo* trials of the antibiotic may not be fruitful.

ACKNOWLEDGEMENTS

REFERENCES

Boorsma, W. G., (1904). Pharmakologische Mitteilung. *Bull. Inst. Bot. Buiten.*, **21**, 1–36.

Bose, P. K. (1958). On some biochemical properties of natural coumarins. *J. Ind. Chem. Soc.*, **35**, 367–375.

Cavallito, C. J. and Bailey, J. H. (1944). Allicin, the antibacterial principle of *Allium sativum* I. Isolation, physical properties and antibacterial action. *J. Amer. Chem. Soc.*, **66**, 1950–51.

Chakraborty, D. P., Das Gupta, A. and Bose P. K. (1957). On the antifungal action of some natural coumarins. *Ann. Biochem. Exptl. Med.*, **17**, 59–62.

Chatterjee, N. G. and Gupta, A. C. (1937). Oil of Nageswar (Iron Wood) seed (*Mesua ferrea*). *Oil. Col. Trades J.*, **91**, 1656.

Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956). Glossary of Indian Medicinal Plants, p. 166. Council of Scientific and Industrial Research, New Delhi, India.

Dhingra, D. R. and Hilditch, T. P. (1931). The fatty acids of some Indian seed oils. The seed fats of *Mesua ferrea*, *Calophyllum inophyllum* and *Pistacia vera*. *J. Soc. Chem. Ind.*, **50**, 9T.

Dutt, P., Deb, N. C. and Bose P. K. (1940). A preliminary note on mesuol, the bitter principle of *Mesua ferrea*. *J. Ind. Chem. Soc.*, **17**, 277–279.

Frost, B. M., Valliant, M. E., McClelland, L., Solotorovsky, M. and Cucklel, A. C. (1955–56). The antimicrobial activity of cathomycin, a new antibiotic. *Antibiotics Annual*, 918–923.

Grimme, Cl. (1910). Über Einige Seltene Ölfruchte, *Chem. Rev. Fett. u. Harz. Ind.*, **17**, 156–158.

Hooper, D. (1908). Notes on Indian drugs. *Pharm. J.*, **27**, 161.

Kaczka, F. J., Shunk, C. L., Richter, J. W., Wolf, F. J., Gasser, M. M. and Folker, K. (1956). Novobiocin III. Cyclonovobiocic acid, a methyl-glycoside and other reaction products *J. Amer. Chem. Soc.*, **78**, 4125–4127.

Kirtikar, K. R. and Basu, B. D. (1935). Indian Medicinal Plants Vol. I. p. 274. Published by L. M. Basu, 49 Leader Road, Allahabad, India.

Ormancey-Potier, A., Buzas, A. and Lederer, E. (1951). Calophyllolide and calophyllic acid from the seeds of *Calophyllum inophyllum*. *Bull. Soc. Chim. Fr.*, 577–580.

REPRODUCTION IN *CLEVELANDIA IOS* (JORDAN AND GILBERT), WITH AN ACCOUNT OF THE EMBRYONIC AND LARVAL DEVELOPMENT

by R. RAGHU PRASAD [1]

(Communicated by N. K. Panikkar, F.N.I.)

ABSTRACT

The paper deals with the size at first maturity, breeding habits and sexual dimorphism, embryonic development and larval stages of the goby, *Clevelandia ios*.

It was noticed that 23 per cent of females start maturing at a standard length of 29.0 mm. The percentage increases gradually and all females 34.0 mm. and above are mature.

The spawning season extends over a period of nine months with the heaviest spawning taking place from March to June. The fish lays about 750 to 1,000 eggs but actual counts of ripe ovarian ova vary from 800 to 1,200 according to the size of the fish.

There is no parental care among *Clevelandia* and the eggs are laid over a considerable area either singly or in small groups. Sexes can be separated easily, in specimens larger than 19 mm. by the examination of the genital papilla. There is no significant difference in standard length between the two sexes, but the length of head and length of maxillary are significantly different in the males and females for a given standard length, they being greater in the males than in the females.

The embryonic development and larval stages of *Clevelandia* up to the tenth day after hatching have been described from eggs artificially spawned in the aquarium. At temperatures varying from 15.0 to 15.5°C the period of incubation extends from ten to twelve days. Descriptions of post larval stages are also given. Attempts to rear the larvae in the laboratory for more than ten days proved to be impossible and the details of the methods tried are given.

INTRODUCTION

The members of the family Gobiidae are exceedingly numerous in the tropical and temperate zones, both in species and individuals, but they are of little economic importance in most parts of the world. This is true largely because most species of this family are small in size. In only one known instance do the members of this family enter the commercial fisheries. The goby fishery in the Philippines is extremely interesting and an account of this has been given by Manacop (1941). Gobies have also been used as bait fish (Weisel, 1947) in the United States of America. Presumably because of their relative economic unimportance no serious attempt seems to have been made to study the complete life history of any species of goby. The author, therefore, undertook to gather as much data as possible on the life history of *Clevelandia ios*, a goby which lives both free and commensally in the burrows of *Urechis, Upogebia* and *Callianassa*. The following account deals only with the size at first maturity, breeding habits, sexual dimorphism, embryonic development and larval stages of *C. ios* [2] and accounts on the other aspects of the investigation on the life history of this goby will be published elsewhere.

The material for this study was collected from Elkhorn Slough, a tributary of Monterey Bay during 1946-48 and the details regarding the locality, methods of

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[2] Recently a series of papers on the life history of Japanese gobies have been published by Dôtu (1954, 1955*a, b* and *c*, 1956*a* and *b*), Dôtu and Mito (1955*a* and *b*) and Dôtu, Mito and Ueno (1955).
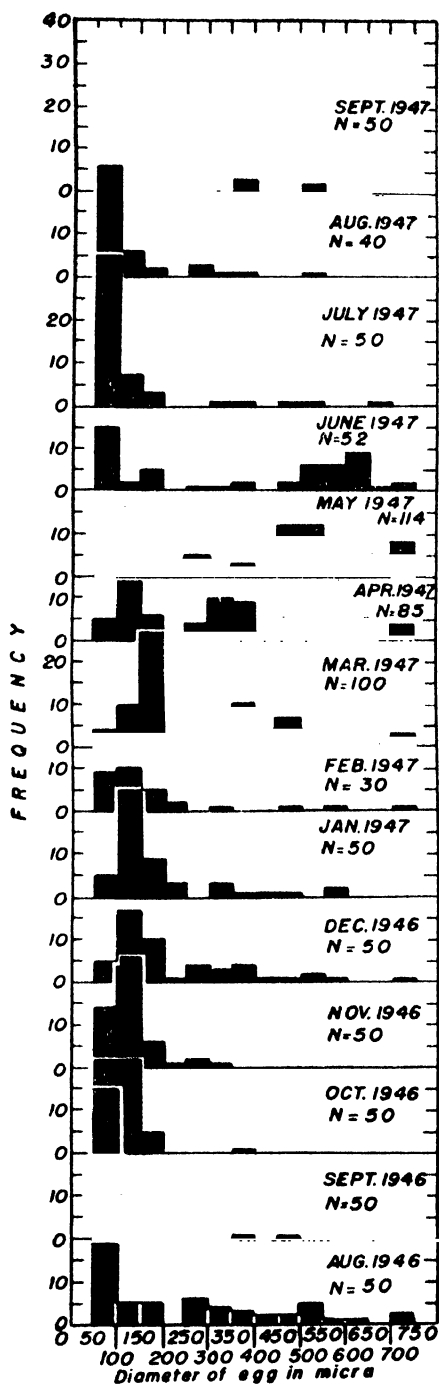
TEXT-FIG. 1.

Ova diameter frequency distribution showing the growth of eggs to maturity.

collection, etc., will be given in the account dealing with the habitat and habits of *Clevelandia*.

SIZE AT FIRST MATURITY

Ovaries were examined from a random sub-sample of each monthly sample in order to follow the growth of eggs. The observations extended from August 1946 to September 1947. In each case the diameter of the egg was measured with the aid of an eye-piece micrometer. The data are given in Fig. 1. In constructing this figure only the largest eggs in the ovary are included and also fish of less than 20.0 mm. are not taken into account. These observations have led to the following conclusions.

A group of eggs measuring up to 200 micra in diameter is seen throughout the year in every adult female. A larger group of eggs, ranging approximately from 250 to 450 micra in diameter, is the maturing group. They start appearing in December and are well represented in the months of March and April ; a few also are found from May to August. A still larger group of eggs, ranging from 500 to 732 micra in diameter, are the ones which are to be spawned immediately. This group is predominant from April to June and is also found in a few individuals in March. It is presumed that the intermediate group of eggs, found in individuals during the peak of the spawning season, will grow to the maximum size by the end of the same season. Since the first group of eggs is present throughout the year in all adult females it is considered to be a group of immature eggs. Moreover, the immature eggs are absolutely transparent with large nucleus (Pl. III, Fig. 1). On the other hand, as the ova start maturing, granules of yolk begin to appear in the cytoplasm and the transparency of the eggs is gradually lost.

Before considering the question of the size at first maturity, it is advisable to define as to what is meant by "mature" and "immature". The word "immature" is sometimes used to refer to young fish which have never spawned and also to designate older fish which have spawned previously but as yet show no indications of the onset of maturity for the next spawning season. Similarly, the word "maturity" has a double meaning.

In this report, fish which have never spawned are designated as "immature". "Maturing" refers to individuals having eggs larger than 200 micra but not yet developed to a stage which is ready for spawning. Mature individuals are those having completely ripe eggs which are ready for liberation. Such mature fish have eggs ranging from 715 to 732 micra in diameter.

The limit of 200 micra is thus fixed to separate the immature from the maturing eggs. Any increase in size beyond this point is regarded as an indication of the beginning of growth toward maturity. The size at first maturity was determined from the data collected during March 1947 to June 1947 (Fig. 2). The percentage of females maturing in each length unit is given in Table 1. For these calculations, data at the onset and the close of the spawning season have been omitted because of the rather irregular and sporadic cases of spawning taking place before and after the maximum spawning period. Thus the data computed is from the heaviest spawning time.

From these data it is concluded that all females smaller than 29.0 mm. in length are immature and that 23 per cent of the fish are found maturing in the 29.0 mm. group ; approximately 39 per cent in the 30.0 mm. group ; 50 per cent in the 31.0 mm. group ; about 62 per cent in the 32.0 mm. group ; about 91 per cent in the 33.0 mm. group and all the females at and above 34.0 mm. are found maturing.

SPAWNING SEASON

From the data presented graphically in Fig. 1 it is clear that the peak of the spawning season extends from March to June ; it is chiefly during these months

Diameter of egg in micra

51-100 101-150 151-200 201-250 251-300 301-350 351-400 401-450 451-500 501-550 551-600 601-650 651-700 701-750

△ March 1947
○ April 1947
✕ May 1947
● June 1947

Standard length in millimeters

0   25   26   27   28   29   30   31   32   33   34   35   36   37   38   39   40   41

2

TEXT-FIG. 2.

Genital papilla

Genital papilla

3

4
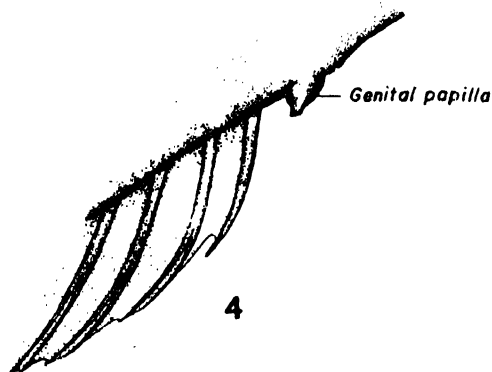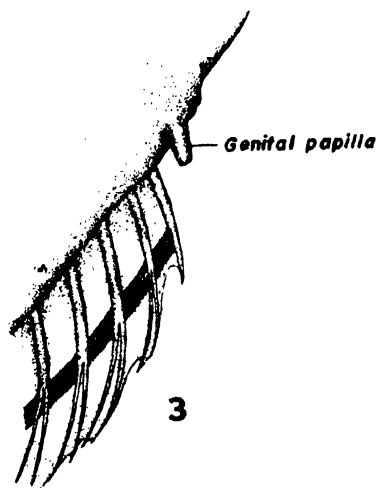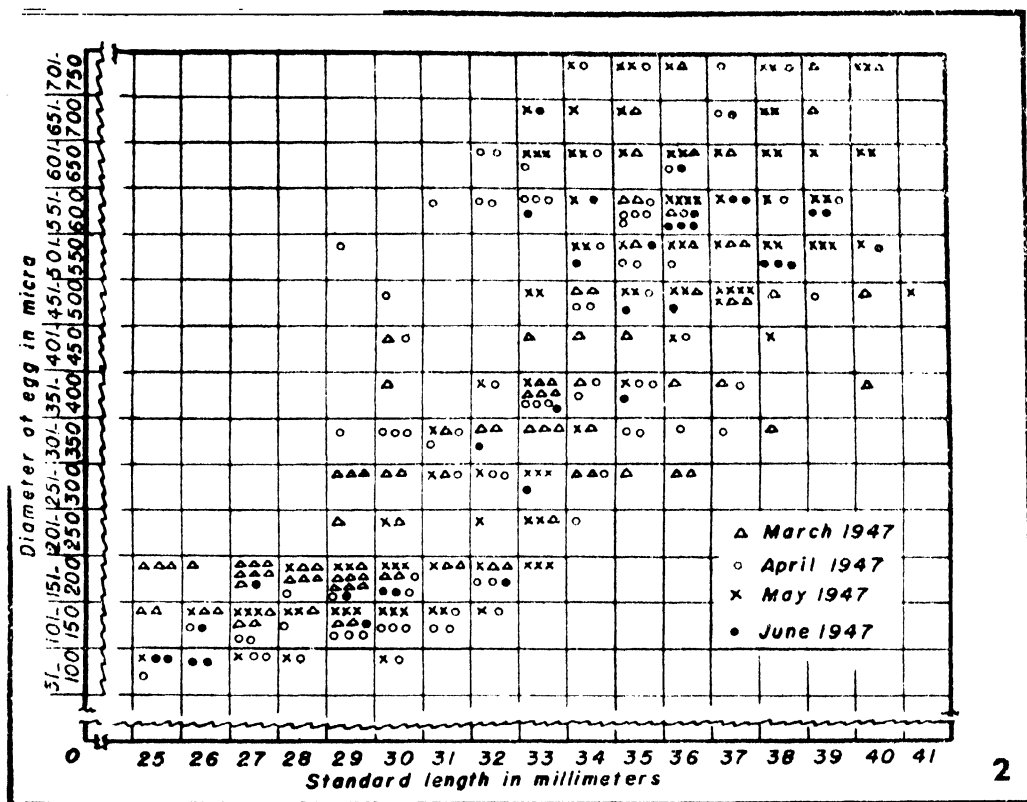
Fig. 2. Distribution of different size groups of eggs in each millimeter of standard length. Class designated as '25' includes all measurements recorded as 25.00, 25.25, 25.50 and 25.75 mm.

Fig. 3. The genital papilla and the black pigment band on the anal fin of a male *Clevelandia*

Fig. 4. The female genital papilla.

TABLE 1

*Number and percentage of fish maturing in each millimeter of body length.
Data collected from March 1947 to June 1947.*

Body length in millimeters[1]	Total numbers	Number maturing	Percentage maturing
25.00	9	0	0
26.00	8	0	0
27.00	19	0	0
28.00	13	0	0
29.00	26	6	23.0
30.00	28	11	39.3
31.00	16	8	50.0
32.00	21	13	61.9
33.00	36	33	91.4
34.00	26	26	100.0
35.00	31	31	100.0
36.00	31	31	100.0
37.00	21	21	100.0
38.00	17	17	100.0
39.00	12	12	100.0
40.00	9	9	100.0
41.00	1	1	100.0

[1] Class designated as "25.00" includes all lengths recorded 25.00, 25.25, 25.50, and 25.75 millimeters.

that mature eggs are found in the ovaries of the females. Apparently, however, some spawning takes place both before and after the maximum period because females with mature or maturing eggs are observed in December, January and February and also in July and August. In Fig. 1 the frequency distribution for August 1946 indicates that there were quite a number of spawners, whereas that of August 1947 shows no individuals containing eggs more than 500 micra in diameter. This discrepancy is believed to be apparent rather than real. Fig. 2 shows that the size of the eggs during the spawning season depends also on the length of the individual fish. It is probable, therefore, that the difference in the relative numbers of mature and immature fish in 1946 and 1947 is due to a difference in the collecting technique—that for 1946 selecting the larger individuals.

An effort was made to locate naturally spawned eggs in the field as an indication of the spawning season but without conclusive results. A few eggs were collected in May 1947 at the height of the spawning season as indicated by the study of ovarian eggs.

The fish kept in the aquarium started spawning in March 1947 ; the first one spawned on 20th March. The second one, which was ready to spawn, was stripped on 8th April and the eggs were artificially fertilized to study the development. A third female spawned on 1st May. Occasional plankton tows were made in the Slough with a view to collecting larvae. A group of twenty larvae, measuring 4.75 mm. in total length and 4.55 mm. in standard length, were collected on 7th July 1947. In general appearance these larvae resemble the larvae reared in the laboratory although there is a slight difference in the arrangement of the pigment pattern. This is presumably due to the fact that the ones taken in the plankton tow are of a slightly more advanced stage of development than those reared in the laboratory. Unfortunately it proved to be impossible to rear those taken from the Slough until they can be positively identified ; nor was it possible to rear the larvae that hatched in the laboratory to a stage comparable to those taken in the Slough. Since there is no other goby found commonly in large numbers in the Slough, it seems safe to assume that these larvae are of *Clevelandia*

ios in spite of the minor differences in pigment pattern, which, as already mentioned, may very well be due to the difference in the stage of development.

On 9th March 1948 five larvae of *C. ios*, ranging in size from 4.25 to 4.50 mm. in length were collected from the Slough with the aid of a plankton net.

From these results it is concluded that *Clevelandia ios* has a protracted spawning season, extending over a period of possibly nine months, but with the heaviest spawning taking place from March to June. Isolated cases of spawning apparently occur as early as December and as late as July and August. There is only a short period, at the most three months, September, October and November when there is no spawning.

Clevelandia lays from 750 to 1,000 eggs at a time. The number of ripe ovarian ova, as shown by actual counts, varies from 800 to 1,200. The size of the ovaries, as well as the number of eggs they contain, varies with the size of the fish. The largest mature ovaries examined (15.0 mm. long and 5.25 mm. wide) were from a fish 50.5 mm. in standard length and contained 1,200 mature ova.

An examination of the ripe ovary reveals eggs of three different sizes (Pl. III, Fig. 2). The largest eggs range in size from 715 to 732 micra while the intermediate group measures between 200 and 250 micra and the smallest from 30 to 95 micra in diameter. Many other species of fish show more than one distinct size of eggs in the ovary (Calderwood, 1892 ; Thompson, 1914 and Clark, 1925 and 1934). These different classes of eggs may indicate a multiplicity of spawning as demonstrated by Clark (1925 and 1934) in *Leuresthes tenuis* and *Sardinops caerulea* respectively or the intermediate and the small eggs may be resorbed after the spawning season as happens in many species of fish. An examination of the cross section of a spent ovary of *Clevelandia* soon after spawning does not show any indication of degeneration, whereas that of a later stage shows signs of disintegration indicating thereby that the unspawned ripe eggs degenerate and are resorbed. The group of intermediate eggs also seems to degenerate while the group of smallest eggs seems to remain unchanged (Pl. III, Fig. 3). This does not mean that the possibility of a multiplicity of spawning in *Clevelandia* may be completely excluded.

BREEDING HABITS AND SEXUAL DIMORPHISM

MacGinitie (1935, p. 748) remarks, "*Clevelandia* lays eggs in the spring, fifteen to twenty-five eggs at a laying. The eggs, which are laid singly, are allowed to settle to the bottom, where they adhere to the sand. The eggs are 735μ long and 570μ wide ; the yolk is 645μ long and the same width as the whole egg." This is all that has previously been known about the breeding habits and eggs of this fish. Great difficulty was experienced in making observations of their breeding habits in the field and hence the following observations were made on specimen, kept in the aquarium.

The females during the breeding season display slight changes in their colour pattern. This is especially marked just a few days prior to the spawning time. This change is purely transitory and disappears soon after spawning. The mature female, ready to spawn, can be easily recognized by its greatly distended abdomen (due to the enormous increase in the size of the eggs) and a bright yellow colour, caused by the underlying eggs, is visible through the distended abdominal wall. The fish becomes sluggish and rather inactive. In some of the mature females it is observed that a streak of black pigment developes on the anal fin. In addition to this there is a great increase in the melanophores on the two dorsal fins and a considerable increase in the number of xanthophores all over the body.

In the males there is as much colour change during the breeding season as in the females. A black streak appears on the anal fin of all mature males. This fades out to a faint streak after milting. Yellow pigments develop chiefly on the ventral surface and two streaks of melanophores appear on the lower jaw. The

melanophores on the dorsal fins increase in number and the upper half of the pectoral shows black pigmentation. Contrary to the behaviour of the females, the males are quite active during the spawning time. There is no distension of the abdominal wall in the males as the testes are extremely small, measuring about 3.0 mm. in length and 0.75 mm. in breadth.

Parental care, in various forms, is a trait often observed amongst gobies and the males or the females, or both, protect the eggs during the period of incubation. *Clevelandia* shows no sign of parental care. This is probably explained by the fact that it lays eggs over a considerable area, either singly or in small groups,[1] whereas those forms which provide parental care deposit their eggs at one place and are generally found attached to some foreign material, such as empty molluscan shells, pieces of wood, etc.

Many species of gobies exhibit marked sexual dimorphism and characters associated with sex have been mistakenly considered as specific. *Clevelandia* does not exhibit striking sexual dimorphism and there are no easily noticeable secondary sexual characters. As already pointed out, mature females can be distinguished from males by the nature of their belly, considerably distended and yellowish in the females. The dark band of pigments on the anal fin of the male is not always a reliable character since it is observed in some of the females, too. However, as in many other gobies, the shape of the genital papilla is a character that can be used with confidence to separate the sexes. In both sexes the papilla is minute and can be differentiated only with the help of a low-power microscope. In the males the genital papilla is straight and tubular (Fig. 3) whereas in the females it is a fleshy bulbous tubercle with a short spout-like opening (Fig. 4). This character, even though readily separates the sexes in the larger fish, cannot be used for those of about 19.0 mm. or less in standard length, in which the difference is not generally marked.

There is no significant difference in standard length between the two sexes. This was determined by a study of the larger fish, above 19.0 mm. in standard length, taken in two collections. A sample collected in October 1946 contained 258 males and 350 females greater than 19.0 mm. in length. Another taken in November 1946 had 273 males and 424 females. The sexing was done by the examination of the genital papilla and in several of the smaller fish this was checked by the examination of the gonad. In the October sample the arithmetic mean (with its standard error) for the males is 26.607 $\pm$ 0.191 mm. and for the females 26.677 $\pm$ 0.175 mm. In the November sample the corresponding figures for the males are 25.673 $\pm$ 0.236 mm. and for the females 25.945 $\pm$ 0.207 mm. The *t*-test was applied to each sample after calculating the standard error of the difference between the means. In both samples the difference in the standard length of the two sexes is not statistically significant, $P > 0.05$.

Other characters such as the length of head, length of maxillary, length and width of snout were also studied for possible differences. For these studies a total of fifty specimens were measured from each sex, their standard length ranging from 20.0 mm. to 40.0 mm. Scatter diagrams were prepared for these—the respective characters plotted against the standard length (Figs. 5, 6, 7 and 8). Both males and females show a very high positive correlation in all these characters and except in one, the length of head, the differences between the correlation coefficients are not statistically significant (Table 2). Even the significance of the difference in

[1] In a personal communication from Dr. Bolin on 17th January 1955, he writes that zoology students from the University of California "found goby eggs, undoubtedly those of *Clevelandia*, attached to the mucus-cemented sand just inside the mouth of the burrow of *Urechis*. I have looked for more on the couple of occasions that I have taken my class over to Elkhorn Slough but have had no luck."

the correlation coefficients of the length of head is rather doubtful as P is greater than 0.02. These tests were carried out by the z-test of R. A. Fisher as given by Simpson and Roe (1939, p. 242).

TABLE 2

Data for the test of significance of the difference between the correlation coefficients

Character studied	♂		♀		σd_z	t	P
	r	z	r	z			
Length of head	+0.973	2.15	+0.989	2.60	0.206	2.18	>0.02
Length of maxillary	+0.993	2.80	+0.989	2.60	0.206	0.97	>0.05
Length of snout	+0.966	1.82	+0.949	2.03	0.206	1.01	>0.05
Width of snout	+0.965	2.01	+0.973	2.15	0.206	0.68	>0.05

In an attempt to ascertain whether any of the characters considered above show sexual dimorphism the significance of the difference between the two regression coefficients for each character of the two sex groups was tested adopting the method given in Simpson and Roe (1939, p. 274).

The given values of P (Table 3) indicate that the differences in the regression coefficients of length of head and length of maxillary are significant whereas those of the length of snout and its width are not. This means that in *Clevelandia ios*, within the range of size studied, length of head and maxillary of the males for a given standard length are significantly greater than those of the females (Figs. 5 and 6). The length of snout and width of snout, on the contrary, are not significantly different (Figs. 7 and 8).

TABLE 3

Data for the test of significance of the characters compared in the males and females of Clevelandia ios.

Character studied	♂ r	♀ r	♂ $b_{y \cdot x}$	♀ $b_{y \cdot x}$	σd_b	t	P
Length of head	+0.973	+0.989	0.296	0.250	0.011	4.22	<0.01
Length of maxillary	+0.993	+0.989	0.227	0.195	0.006	5.32	<0.01
Length of snout	+0.966	+0.949	0.083	0.093	0.005	1.76	>0.05
Width of snout	+0.965	+0.973	0.172	0.158	0.009	1.62	>0.05

EMBRYONIC DEVELOPMENT AND LARVAL STAGES

The development of *Clevelandia ios* up to the tenth day after hatching was followed in the eggs spawned artificially on 8th April 1947. The eggs were stripped into a clean finger bowl which had been rinsed with fresh sea water. Owing to

the difficulty experienced in stripping the males, the testes, removed by dissection, were teased with a pair of dissecting needles. To this were added a few drops of sea water. The sperms were distributed uniformly over the eggs with the aid of an eye-dropper. The bowl was gently stirred to ensure fertilization of all the eggs. After two minutes the eggs were washed several times in fresh sea water to remove the excess of spermatozoa and to prevent polyspermy. The eggs were kept in the laboratory at temperatures varying from 15° to 15.5°C
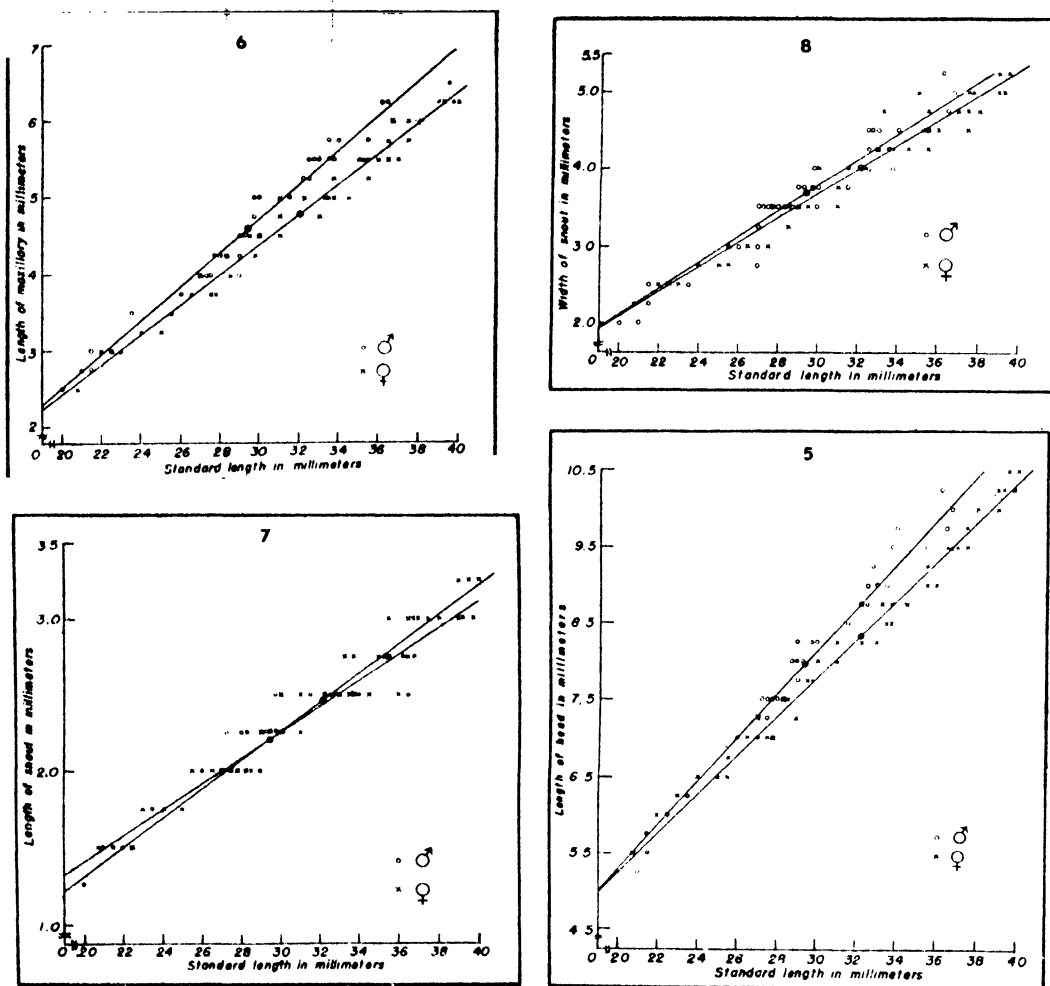
TEXT-FIG. 3.

Fig. 5. The relation between standard length and length of head in the males and females of *Clevelandia*.
Fig. 6. The relation between standard length and length of maxillary in males and females.
Fig. 7. Length of snout of the males and females plotted against standard length.
Fig. 8. Width of snout of the males and females graphed against standard length.

The unfertilized eggs are honey coloured, translucent and almost spherical, measuring 735 micra in diameter (Fig. 9). As soon as the eggs come into contact with water, the outermost transparent net-like covering consisting of thread-like filaments, breaks at the pole, opposite to the pole of attachment, gradually falls

down forming a circlet of adhesive threads (Fig. 10) which attach the eggs to the substratum. Duncker (1929, p. XII. g. 125) remarks: "Die Eir sind vor ihrer Ablage kugelförmig und von einer netzartig durchbrochenen Membran umhüllet. Bei der Ablage platzt diese an dem einen Pol des sich streckenden Eies, bleibt aber an dem gegenüberliegenden fest, und mittels ihrer ursprünglichen Aussen-fläche heftet sich das abgelegte Ei auf seiner Unterlage fest." But in *Acentrogobius neilli*, Aiyar (1935, p. 84) observed: "At the narrow end the micropyle can be seen as a distinct opening. Streaming out of the opening are filaments which immediately attach the egg-case to the bottom of the glass." A tendency for the threads of the adjacent eggs to remain together is noticed and this results in the aggregation of the eggs into bunches which look like bunches of grapes. Eggs also attach themselves to the substratum singly. They do not show a tendency to float. In the Slough the eggs of *Clevelandia* are found attached to sand grains.

The egg membrane of an unfertilized egg remains very close to the yolk and between it and the yolk there is a barely perceptible perivitelline space. The deli-cate and transparent egg membrane has extremely fine longitudinal striations or the surface. It was not possible to locate the micropyle. The protoplasm is distributed in an almost uniform layer over the surface of the yolk and there are numerous oil globules of different sizes distributed in the yolk.

Blastodisc : As already mentioned, the bulk of the protoplasm at the time of stripping is distributed over the surface of the yolk in a layer of uniform thickness. It begins to concentrate at one pole, the animal pole, within five minutes after fertilization, to form the blastodisc (Fig. 11). This concentration is brought about by the protoplasm flowing toward the animal pole. The pheno-menon of streaming of protoplasm during the formation of the blastodisc has been described in the eggs of various teleostean fishes by Ryder (1884), Wilson (1891), Kuntz (1916), and others. No such streaming of protoplasm can be observed in the species under discussion, probably due to the fact that the protoplasmic granulations are too fine. Kuntz (1916, p. 424) remarks that in the eggs of *Gobio-soma bosci* "The protoplasmic movements involved in the process of concentration can not be satisfactorily observed by reason of the opacity of the yolk." Whereas in the eggs of *Ctenogobius stigmaticus* he says that : "The process can hardly be described as a "streaming" of the protoplasm toward one pole of the yolk sphere, but rather as a thinning of the layer of protoplasm on one side of the yolk and a corresponding thickening on the opposite side which pushes the yolk out into an extremely eccentric position [427]." Even though no streaming of protoplasm is noticed in *C. ios* there is no question about the redistribution of the protoplasmic material which concentrates at one pole. There is not any appreciable shrinkage of the yolk sphere.

Concomitant with these changes there is a gradual stretching of the shell, by absorption of water, thus increasing the perivitelline space. The eggs now assume an almost elliptical form. The yolk with the growing blastodisc remains practically at the centre of the capsule (Fig. 12).

About two hours after fertilization the egg-case reaches its maximum size, 1,300 micra along the major axis and 790 micra along the minor axis. Slight variations are observed in the size of the egg-cases. The smallest was 1,170 micra by 715 micra while the largest measured 1,300 micra by 790 micra. Thus there is an enormous increase along the major axis but the minor axis hardly increases at all. At this stage the blastodisc is fully developed and appears as a symmetrical dome-shaped cap on the yolk sphere. The fully differentiated blastodisc is fairly thick and by about two hours and fifteen minutes it is quite sharply defined. The amount of protoplasm in relation to the yolk is considerable. In 95 per cent of the eggs the blastodisc is toward the attached end of the egg whereas in the remaining 5 per cent it is on the side away from the base of the egg-capsule. At this stage

there is no visible change in the size or shape of the yolk-mass except that at the top it is slightly flattened (Fig. 13). The oil globules remain the same.

Segmentation : The first sign of cleavage starts at approximately two and half hours after fertilization. When cleavage starts, in most cases, the yolk with the blastodisc migrates gradually to the distal end of the egg capsule (away from the attachment). In a few of the eggs this process may start a little later and consequently the yolk with the dividing blastodisc reaches the narrow end of the egg-case only when it is in the eight- or sixteen-cell stage. The oil globules show a tendency to accumulate under the blastomeres. By the end of three hours the first cleavage is completed resulting in two huge blastomeres (Fig. 14 and Pl. III, Fig. 4). The first cleavage plane is meridional and divides the blastodisc into two blastomeres equal in size and which, when viewed from above, appear almost circular in outline. This results in the axis of the blastoderm being elongated at right angles to the cleavage plane. The subsequent cleavages take place at hourly intervals. The second cleavage which is also meridional and at right angles to the first, results in four equal blastomeres standing out as more or less isolated rounded elevations (Fig. 15 and Pl. III, Fig. 5). The four blastomeres are still large and as a result of the second cleavage the axis of the blastoderm is restored to symmetry.

The third cleavage, resulting in two parallel rows of four symmetrical blastomeres, takes place on each side of, and parallel to, the first plane of cleavage. At the end of this cleavage the axis of the blastoderm is once again lengthened and a marked diminution in the size of the individual blastomeres is observed (Fig. 16 and Pl. III, Fig. 6).

The result of the fourth cleavage, resulting in sixteen cells, is that the blastoderm once again becomes almost circular in outline. As cleavages advance not only is the size of the individual blastomeres reduced but they exhibit a great degree of irregularity in size, shape and position. With the fifth cleavage and the formation of thirty-two cells the blastoderm becomes two cells in thickness and from here on it is practically impossible to follow the course of cleavage in the living material. The size of the yolk-mass is reduced gradually with the onset of further cleavage.

After nine hours the blastoderm has assumed the form of a thick dome-shaped cap with sharply defined edges but still the individual cells can be made out under the microscope (Fig. 17). As development proceeds it becomes more and more difficult to distinguish the individual cells as they become progressively smaller until finally at the end of about twenty-one and half hours the blastoderm somewhat resembles in appearance the stage just before the first cleavage took place except that the dome-shaped blastoderm has already begun to lose its steepness and to thin out as it spreads around the yolk (Fig. 18).

Segmentation cavity : The segmentation cavity appears soon after the blastoderm has assumed the stage when the individual cells are indistinguishable. about twenty-two hours after fertilization. It first becomes visible as a narrow central area beneath the blastoderm and in about twenty-four hours the cavity is well represented (Fig. 19). In the living eggs of *Clevelandia ios* it is impossible to make out the periblast. According to Kuntz (1916) the periblast of the eggs of *Gobiosoma bosci* appears relatively thick. but cannot be satisfactorily observed in the living material by reason of the opacity of the yolk. In the early stages of the formation of the segmentation cavity the solid cellular wall is of uniform thickness. As growth proceeds the roof of this cavity becomes thinner, while the cavity itself becomes larger, but narrower and spreads more over the yolk-mass. The margin of the blastoderm grows down around the yolk-mass. Due to this growth the yolk-mass, covered over by the blastoderm, slightly elongates (Fig. 20 and Pl. III, Fig. 7). As a result of the centripetal migration of the peripheral cells of the blastoderm, a thickened cellular rim is formed around the edge of the blastoderm.
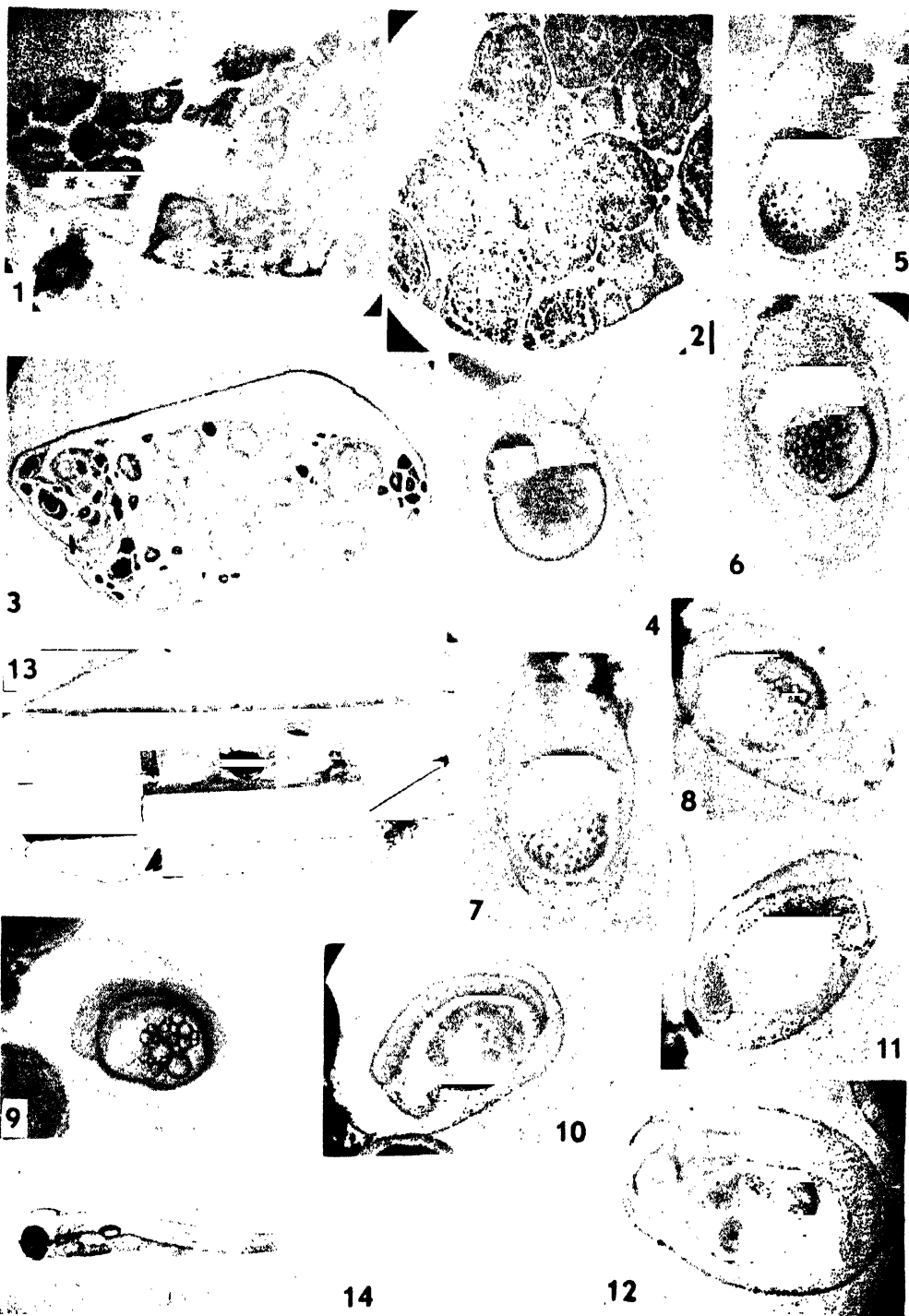
This is the germ ring which, until the closure of the yolk blastopore, marks the advance of the blastoderm. The yolk-mass at the level of the germ ring is slightly constricted.

This growth by the proliferation of the marginal cells continues uniformly around the yolk until the blastoderm covers almost three quarters of the surface of the yolk-mass. But with the appearance of the germ ring the proliferation of the peripheral cells, which resulted in the formation of the germ ring, is much more rapid at one pole than around the rest of the margin of the blastoderm. This place denotes the posterior pole of the future embryo. An extremely rapid proliferation of cells occurs here resulting in the formation of a broad tongue which grows forward into the segmentation cavity. This is the embryonic shield. Along the median line of the embryonic shield a thickening soon appears (Fig. 21) which represents the axis of the future embryo and this comes to lie parallel to the major axis of the egg-capsule. The thickening referred to above soon grows forward (Fig. 22 and Pl. III, Fig. 8) and projects into the perivitelline space. It produces a deep V-shaped groove in the yolk, in which the early embryo lies cradled.

The yolk blastopore remains open until forty-one hours after fertilization but in another hour it is completely closed. At about the same time the blastopore is closed, the embryo extends about three quarters of the way around the remaining yolk-mass and the V-shaped groove has deepened considerably. Kupffer's vesicle has put in its appearance and is seen embedded in the yolk at the posterior end of the embryo (Fig. 23).

The formation of the optic vesicle and the appearance of the first pair of mesoderm somites occur almost simultaneously at about forty-six hours. The first pair of somites appear almost near the middle of the embryo (Figs. 24, 25 and Pl. III, Fig. 9). Along with these changes the oil globules gradually decrease in number but at the same time there is a tendency for those remaining to become larger. This is brought about by the fusion of two or more oil globules. By now the yolk-mass presents a granular appearance.

About fifty-nine hours after fertilization the embryo has developed considerably. The optic cup, well developed and with a prominent choroid fissure, has increased in size. The auditory capsules have made their appearance as a pair of small oval vesicles posterior to the eyes. The notochord can be seen and there are ten fully formed somites. Kupffer's vesicle is still persistent but has been reduced in size (Fig. 26 and Pl. III, Fig. 10). Very soon the tail of the embryo shows indications of getting detached from the underlying yolk-mass. This is very well seen in Pl III, Fig. 11. The quantity of yolk has been reduced considerably and the yolk appears to be more opaque. The three primary divisions of the brain can be made out. The number of oil globules is reduced to two or three. In most cases the embryo lies with the head toward the free end of the egg-capsule but a few are found to be the other way about.

About sixty-three hours after fertilization Kupffer's vesicle has completely disappeared and shortly after this the tail becomes free from the yolk. The eyes are now provided with lens. In another six hours the pericardial cavity appears. Growth takes place rapidly at the tail region and there are fourteen fully formed somites.

The embryo has completely filled the egg-capsule by about seventy-one hours. The tail is still blunt and bent downwards. On the ventral side of the embryo where the tail bends, a pit is seen which is the anal pit. A few cells can be made out in the pericardial cavity and soon indications of the formation of the heart are seen. At about the same time the olfactory capsules appear as a pair of horseshoe-shaped depressions below the eyes. In another six hours the tail, still bent downwards, has grown down further and the tip is in level with the tip of the head. The head has slightly increased in size and some of the lobes of the brain can be made out. The heart is seen as a simple tube containing a few corpuscles. The

otoliths are clearly visible, so is the posterior part of the alimentary canal (Fig. 27 and Pl. I, Fig. 12).

About eighty hours after fertilization the heart is seen pulsating slowly.   There remains a single oil globule in the yolk.

The growth proceeds rapidly and by the fifth day the embryo has begun to move in the egg-case.   It rotates on its longitudinal axis ; twitches its tail, and exhibits wriggling movements. The yolk has become practically spherical. As the embryo lengthens, the tail becomes more active and moves constantly.   It is kept bent either to the right or to the left side of the embryo.   The fin fold develops as a continuous fold running from the nape around the tail to the posterior margin of the yolk-mass (Fig. 28).

Pigmentation in the eyes appears very early on the sixth day and the eyes become increasingly darker as development progresses. About six hours after pigmentation first appears in the eyes, melanophores can be observed developing along the dorsal and ventral sides of the posterior half of the body.   They appear as small black dots along the region where the fin fold and the body meet.   By now the tip of the tail (which is bent forward) extends as far as the middle of the eye and the rudiments of the pectoral fins are visible. The fin fold has become slightly wider (Fig. 29).

The stomodaeum becomes apparent by the seventh day.   In the embryo of the eighth day the eyes have the silvery sheen.   Four stellate melanophores appear on the yolk-mass. The quantity of yolk has been considerably reduced and the yolk-mass is about 455 micra in diameter.   There is the first indication of the air bladder which appears just above the yolk-mass at the level of the pectoral fin. The embryo exhibits occasional violent jerking movements.

On the ninth day the pectoral fins are well developed and the embryo has started using them actively.   Pigmentation has increased on the yolk-mass.   The air bladder is very well seen and pigments have developed on them.   The movements of the embryo are more vigorous.   By the end of the ninth day the melanophores have become larger.   The embryo has by now a well developed mouth.

As the tenth day approaches the embryo becomes more and more active. The yolk-mass is reduced to about 325 micra in diameter and there is still one oil

TEXT-FIG. 1.

Fig.   9.   An unfertilized egg after the formation ov the adhesive threads.
Fig.   10.   The circlet of adhesive threads at the base of the egg-capsule.
Fig.   11.   An egg five minutes after fertilization showing the change in the distribution of the protoplasm.
Fig.   12.   An egg showing the elongated egg-capsule and the increased perivitelline space.
Fig.   13.   Egg with fully developed blastodisc.
Fig.   14.   Egg showing two blastomeres.
Fig.   15.   Egg with a blastoderm of four cells.
Fig.   16.   Egg in the eight cell stage.
Fig.   17.   Egg with a many celled blastoderm.
Fig.   18.   Egg showing the dome-shaped blastoderm resembling the blastodisc of Fig. 13.
Fig.   19.   Egg showing the segmentation cavity.
Fig.   20.   Egg with increased segmentation cavity and slightly elongated yolk-mass.
Fig.   21.   Egg showing the embryonic axis.
Fig.   22.   Egg showing an advanced stage in the differentiation of the embryonic axis.
Fig.   23.   Egg showing embryo with Kupffer's vesicle.
Fig.   24.   Embryo with well developed optic vesicle and a pair of mesoderm somites.
Fig.   25.   Dorsal view of the embryo shown in Fig. 24 showing the V-shaped groove in which the embryo lies.
Fig.   26.   Egg with an advanced embryo showing well developed optic cup with choroid fissure, auditory vesicles, notochord and mesoderm somites. Notice the reduction in the size of Kuffer's vesicle.
Fig.   27.   The embryo showing olfactory capsule, tubular heart, anal pit and posterior part of the alimentary canal.

TEXT-FIG 1.

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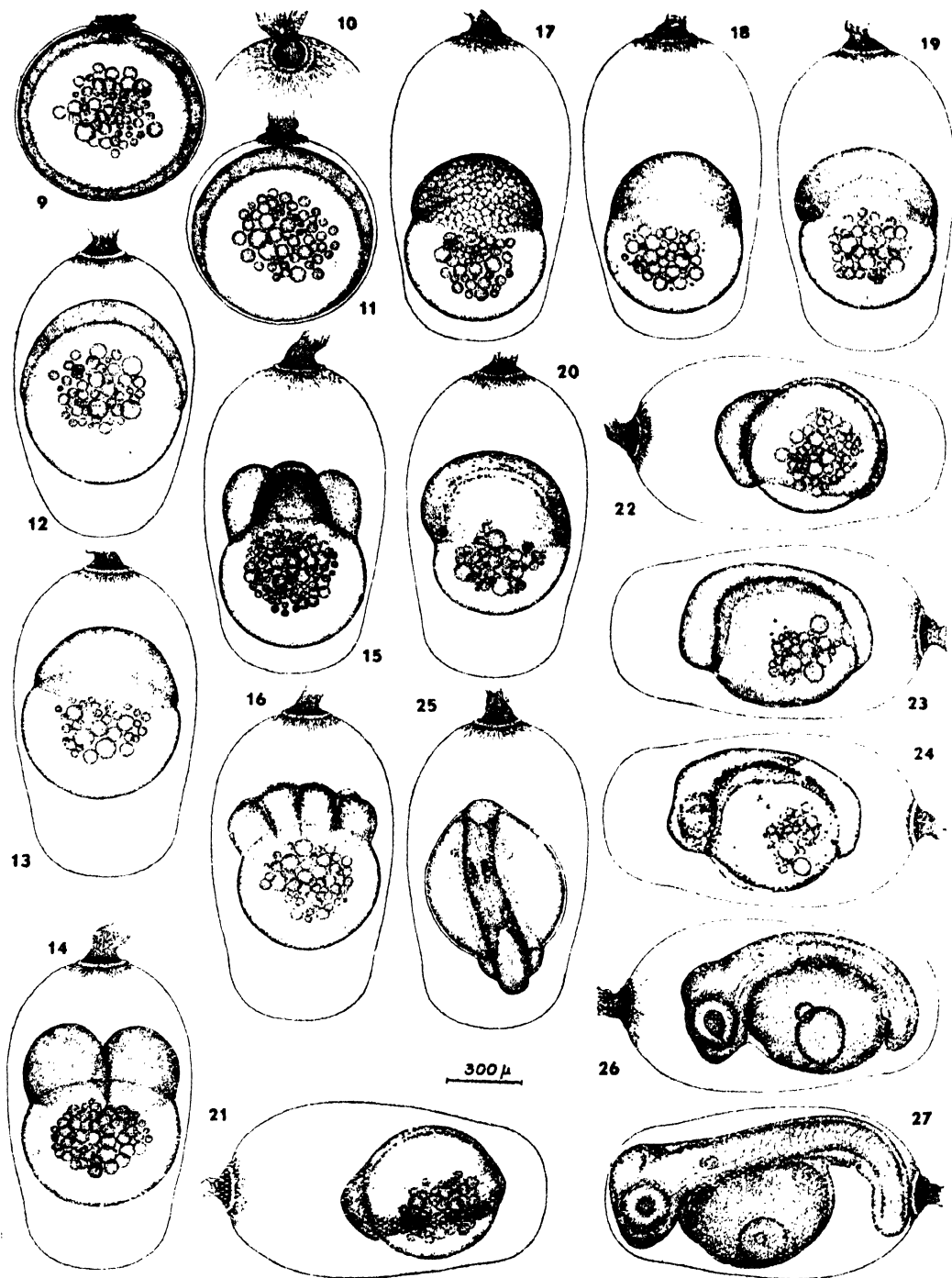
globule persisting ; but it has become much smaller. Along the dorsal side can be seen three melanophores, the last one being the largest. On the ventral side are two rows of melanophores, one along the edge of the body, composed of about eleven stellate melanophores and the other on the lower part of the alimentary canal, composed of about six small pigment spots. On the yolk can be made out about seven melanophores. The arrangement and number of melanophores seem to be fairly constant for the species. Associated with the fourth and eighth melanophores on the ventral side are two xanthophores. A series of xanthophores extend from the region of the mid brain up to the last melanophore on the dorsal side. But these xanthophores seem to be transitory. On careful examination corpuscles can be seen moving in the blood vessels but they are still colourless. On the evening of the tenth day, 228 hours after fertilization, the first larva hatched. A series of jerking, wriggling and lashing movements of the embryo rupture the egg membrane. The tail comes out first in most cases and gradually the larva wriggles out of the egg-case. The time of hatching varies considerably. The first one hatched at 9 p.m. on the 17th April and the last one hatched on the 19th at 11 a.m.

Larvae : At temperatures varying from 15° to 15.5°C the incubation period extends from ten to twelve days. This seems to be in agreement with the incubation period of many other species of gobies, both American and European. For the blind goby, *Typhlogobius californiensis*, the incubation period is ten to twelve days at temperatures ranging from 17° to 20°C (MacGinitie, 1939). Weisel (1947) assumes that at about 18°C the period of development prior to hatching for *Gillichthys mirabilis* is ten to twelve days. For those European species such as *Gobius microps*, *G. minutus* and *G. pictus*, Shann (1910) mentions that the period of incubation is about fourteen days. But according to Kuntz (1916) the incubation period of *Gobiosoma bosci*, an American species, at "laboratory temperature" is approximately five days whereas for *Ctenogobius stigmaticus* the period of incubation is not over eighteen hours.

The newly hatched larvae of *Clevelandia ios* (Figs. 30 and 31) are pelagic. They vary in size from 2.75 mm. to 3.25 mm. in total length. They are slender, delicate and transparent. The head is rounded with well-developed mouth which is horizontal and inferior. The eyes are very prominent. The auditory vesicles and the notochord are clearly seen. The yolk-sac, which is almost round, ranges in diameter from 260 to 325 micra. The oil globule is still present even though reduced in size. The fin fold is rather broad and on the dorsal side it starts from opposite the middle of the yolk-sac and is continuous with the ventral fin fold which stops at the vent. From the vent there is another short fold extending up to the posterior margin of the yolk-sac. The tail is rounded. The vent is situated almost at the middle of the body, being slightly closer to the tip of the tail.

The larvae exhibit a characteristic pattern of pigmentation that is common to many of the larvae of gobies. The xanthophores which were present on the head region have disappeared. All the melanophores which were described in the previous stage are still present. On the dorsal side are three melanophores, the last one being the largest. With it is associated a xanthophore. On the ventral side are eleven melanophores and associated with the fourth and eighth are xanthophores. Along the ventral side of the alimentary canal, at the place of junction of the fin fold and the alimentary canal are distributed six melanophores and usually seven or eight melanophores can be seen on each side of the yolk-sac. The upper part of the air bladder is covered with melanophores and a few xanthophores thus presenting the characteristic crescent shaped black area on the upper half. The larvae are exceedingly active and are positively phototropic.

This detailed description of the larvae is made, in part, to facilitate the identification of the larvae of *Clevelandia ios* that may be taken in nature in future studies.

The larvae survived in the laboratory for ten days. Different methods were tried to keep them alive longer but all attempts failed. They were kept at a fairly constant temperature ranging from 15° to 15.5°C. The water was changed every twenty-four hours. In one lot the water in the bowl was kept gently stirred with the aid of a plunger, while the other lot was left undisturbed. The result was the same in both cases. Different types of food, such as, algal spores, nauplii, sea urchin blastulae and diatoms of different species were provided. Occasionally plankton hauls were made and the various small organisms present in it were also given as food. An examination of these larvae showed that they were not feeding. Their alimentary tracts were completely empty except for one specimen. In another attempt to rear the larvae, six of them were left in a bottle covered with fine bolting silk, which permitted small organisms to get in but at the same time prevented the larvae from escaping. This was left floating on the surface of the sea with the help of a wooden raft. The complete equipment is shown in Pl. III, Fig. 13. This was left approximately 200 yards from the shore. The larvae were periodically examined. Although this method provided the larvae, all the time, with fresh sea water and presumably enough food, all of them died within a period of nine to eleven days. Owing to many difficulties in keeping such equipment in Elkhorn Slough no attempt was made at rearing the larvae in the Slough although it might have proved more successful. Two stages in the growth of the larvae are described below. The larva shown in Fig. 32 is six days after hatching and measured 3.5 mm. in total length. It has undergone only slight changes from the newly hatched larva. The general shape of the head has changed and has become more pointed. The position of the mouth shifted slightly to terminal and more or less oblique. The quantity of yolk has been reduced considerably. The oil globule, even though still persistent, is extremely small. Owing to the more rapid growth of the tail region the vent is much nearer to the head than to the tip of the tail. The tail is still rounded and the continuous fin fold remains broad. Of the three melanophores on the dorsal side, two of the smaller ones have disappeared.

Figure 33 and Pl.III, Fig. 14 show a larva measuring 3.9 mm in total length. No great advance over the preceding stage is shown. The larva is ten days old at which age all of them died. The yolk is completely absorbed. The air bladder has slightly increased in size and so also have the pectoral fins, which the larva uses quite actively. During this period the larva increased approximately 1.2 mm. in total length.

The next developmental stage obtained was post larval, averaging about 7.00 mm. in standard length and 7.75 mm. in total length (Fig. 34). Three of these were collected from Elkhorn Slough, with the aid of a plankton net, on 7th July 1947. The larvae are still transparent. The mouth has become quite oblique and the skeletal elements are fairly well developed. The fish are still transparent enough so that the vertebral column can be seen and thirty-five vertebrae can be counted. Through the body wall the air bladder is still perceptible. All fins except the first dorsal and the ventral fins are well developed. The rudiments of the ventral fins show as light thickenings on the ventral surface immediately below the base of the pectorals. The spinous first dorsal fin is not yet formed. The caudal fin has become truncated and has a straight posterior margin. Twelve rays can be counted in the second dorsal fin, twelve also in the anal fin and fourteen in the caudal fin. The hypural bone and the urostyle can be clearly made out. The membraneous fold of skin on the ventral side in front of the anus has begun to decrease in width. The dorsal fin fold is still continuous with the posterior half of the ventral fin fold, but at certain places it has begun to decrease in width. Yellow pigments are found in association with melanophores especially with the one on the dorsal side and the ventral group behind the anus. Even though the pigment pattern remains basically the same, slight alterations have taken place. The individual melanophores on the ventral side behind the anus have fused to

form a streak whereas those below the alimentary tract still remain separated. There has appeared a pigment spot on the chin below the middle of the eye and the pigments on the caudal fin are clearly seen. The auditory vesicles are still visible from the outside and it is interesting to note that the posterior otolith has grown to be larger than the anterior one. The same condition has been described in the post larval stage of *Chasmichthys gulosus*, measuring 6.7 mm. in length, by Nakamura (1936).
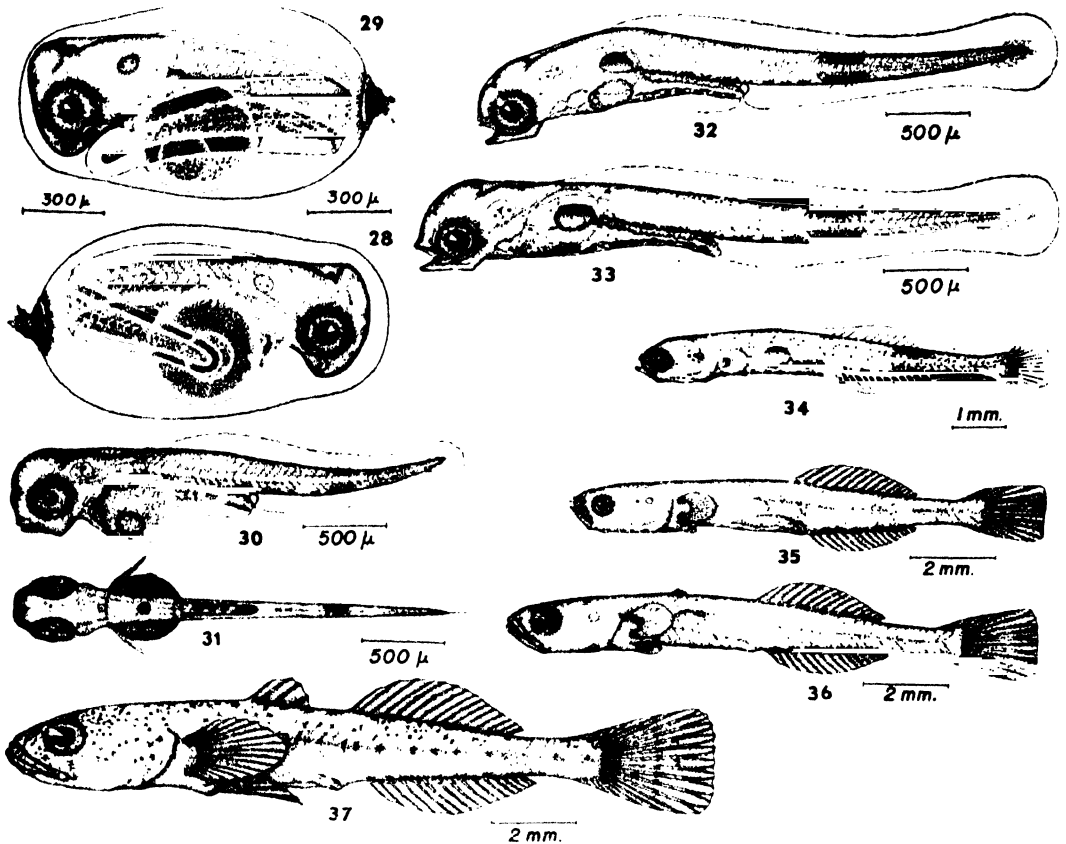
TEXT FIG. 5

Fig. 28.   A well developed embryo showing the fin fold.
Fig. 29.   An advanced embryo with the rudiment of the pectoral fin and melanophores in the tail region.
Fig. 30.   A newly hatched larva.
Fig. 31.   The dorsal view of the newly hatched larva.
Fig. 32.   A larva six days after hatching.
Fig. 33.   A ten day old larva.
Fig. 34.   A post larva 7.0 mm. in standard length.
Fig. 35.   A post larva 9.75 mm. in standard length.
Fig. 36.   A specimen 10.75 mm. showing the development of the first dorsal fin and the two separate ventral fins.
Fig. 37.   A juvenile 14.00 mm. in standard length showing all the essential characters of the adult.

It is not long after this before the adult characters are established. A specimen 9.75 mm. in standard length is shown in Fig. 35. The body is still more or less transparent and such internal structure as the vertebral column and the air

bladder are still clearly visible as in the previous stage. The fin fold which was persistent and continuous in the earlier stage is no longer continuous. The first dorsal fin has not yet developed. It is interesting to note that the fin fold at the place where the first dorsal fin is to appear, is absorbed completely, while at the same time it persists caudad and all the three fins viz., the second dorsal, caudal and anal, are developed from it. The same has been observed in *Chasmichthys dolichognathus* by Nakamura (1936). The fin-rays in the three fins just mentioned are quite definite. The pectoral fins show indications of the development of rays. The ventral fins, however, are still in the form of two buds. The auditory vesicle is no longer clearly visible from the outside.

The pattern of distribution of pigments has changed. There are no pigments on the dorsal margin of the body. Along the ventral side there are two black pigment spots in the head region, about nine from the base of the operculum to anus and about six along the base of the anal fin.

Fig. 36 represents a fish measuring 10.75 mm. in standard length. The important changes from the previous stage are, (1) the appearance of the spinous dorsal fin which has only three spines at this stage (2) the ventral fins have developed further and are visible as two separate fins in close proximity, (3) the eyes have moved slightly dorsally. The remaining characters are almost the same as in the previous stage except that between the ventral fins and the anus there are fewer melanophores.

All of the essential adult external characters are developed in a juvenile measuring 14.0 mm. in standard length (Fig. 37). The fins are fully developed with the characteristic number of fin-rays. The ventral fins have fused to form a single fin. The distribution of the integumentary papillae has assumed more or less the typical form. The eyes have practically moved to the position taken in the adults. Thus, at this stage the species can be identified with certainty and, in appearance, the juveniles resemble the adults except that they are less heavily pigmented.

REFERENCES

Aiyar, R. G. (1935). Observations on the development of *Acetrogobius neilli* (*Gobius neilli* Day). *Zool. Anz.*, **111**, 83–92.

Calderwood, W. L. (1892). A contribution to our knowledge of the ovary and the intra-ovarian eggs in Teleosteans. *Journ. Mar. Biol. Assn.* U.K., **2**, 289–313.

Clark, F. N. (1925). The life history of *Leuresthes tenuis*, an Atherine fish with tide controlled spawning habits. Div. Fish and Game, California, *Fish Bull.*, **10**, 1–51.

———— (1934). Maturity of the California Sardine (*Sardina caerulea*), determined by ova diameter measurements. *Ibid.*, **42**, 5–49.

Dôtu, Y. (1954). On the life history of a goby, *Chaenogobius eastanea* O'Shaughnessy. *Japanese J. Ichthy.*, **3**, 133–138.

Dôtu, Y. (1955*a*). Life history of a goby, *Gobius poecilichthys* Jordan et Snyder. *Sci. Bull. Faculty Agri.*, Kyushu Univ., **15**, 77–86.

— — — (1955*b*). The life history of a goby, *Chaenogobius urotaenia* (Hilgendorf). *Ibid.*, **15**, 367–374.

— — — — (1955*c*). On the life history of a Gobioid Fish, *Eutaeniichthys gilli* Jordan et Snyder. *Bull. Biogeographical Soc. Japan.*, **16-19**, 338–344.

— — — — (1956*a*). On the habits and egg-development of a Goby, *Pterogobius zonoleuscus* Jordan et Snyder. *Sci. Bull. Faculty Agri.*, Kyushu Univ., **15**, 483–487.

— — — (1956*b*). The life history of an Eleotrid goby *Parioglossus taeniatus* Regan. *Ibid.*, **15**, 489–496.

Dôtu, Y and Mito, S. (1955*a*). Life history of a Gobioid fish, *Sicydium japonicum* Tanaka. *Ibid.*, **15**, 213–221.

— — — — (1955*b*). On the breeding-habits, larvae of a Goby, *Acanthogobius flavimanus* (Temminck et Schlegel). *Japanese J. Ichthy.*, **4**, 153–161.

Dôtu, Y., Mito, S. and Ueno, M. (1955). The life history of a Goby, *Chaeturichthys hexanema* Bleeker. *Sci. Bull. Faculty Agri.*, Kyushu Univ., **15**, 359–365.

Duncker, G., *et al.* (1929). Die fische der Nord-und Ostsee. Akademische Verlagsgesselschaft, Leipzig.

Kuntz, A. (1916). Notes on the embryology and larval development of five species of teleostean fishes. *Bull. U.S. Bur. Fish.*, **34**, for 1914, 407–429.

MacGinitie, G. E. (1935). Ecological aspects of a California marine estuary. *Amer. Mid. Nat.*, **16**, 629–756.

Manacop, P. R. (1941). The life history and habits of the goby, *Sicyopterus extraneus* Herre (Anga), Gobiidae, with an account of the goby fry fishery of Cagayan River, Oriental Misamis Province, Mindanao, Philippines. Thesis submitted to the School of Biological Sciences and the Committee on Graduate Study of the Stanford University in partial fulfillment of the requirements for the degree of Master of Arts.

Nakamura, S. (1936). Larvae and young of fishes found in the vicinity of Kominata. II-VI. *Imp. Fish. Inst.* Tokyo, **31**, 131–166.

Ryder, J. A. (1884). A contribution to the embryology of osseous fishes, with special reference to the development of the cod (*Gadus morrhus*). Rept. U.S. Comm. Fish. for the fiscal year 1882, Pt. 10, 455–605.

Shann, E.W. (1910). Some notes on the life-history and rate of growth in *Gobius minutus*. *Ann. Mag. Nat. Hist.*, 8th Ser., **5**, 217–239.

Simpson, G. G. and Roe, Anne (1939). Quantitative Zoology,, First Ed., McGraw Hill Book Co., New York.

Thompson, W. F. (1914). A preliminary report of the life-history of the halibut. Rept. British Columbia Fish. Dept., 76–122.

Weisel, G. F., Jr. (1947). Breeding behaviour and early development of the mudsucker, a Gobiid fish of California. *Copeia*, No. 2, 77–85.

Wilson, H. V. (1891). The embryology of the sea bass (*Serranus atrarius*). *Bull. U.S. Fish. Comm.*, **9**, 209–277.

STUDIES IN THE ORDER PIPERALES. IV. A CONTRIBUTION TO THE STUDY OF VEGETATIVE ANATOMY OF THREE SPECIES OF *PIPER*[1,2]

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(Communicated by G. P. Majumdar, F.N.I.)

ABSTRACT

Anatomy of the vegetative organs of three species of *Piper*, has been studied. It has been observed that all the three species agree in general anatomical features. The axis is sympodial. Generally more than five leaf traces are present which may or may not be of the same size. Stipules do occur in *P. betle*. *Piper* too is exceptional in having a single prophyll.

INTRODUCTION

The genus *Piper*, after which the family and the order are named, is the best known genus of the family perhaps for its scientific and commercial importance. Much of the published work before the beginning of the present century confined to taxonomy, but in recent years embryology and anatomy too received some attention. Chibber (1913) gave an account of the morphology and histology of the vegetative organs of *Piper betle*. Rousseau (1927) studied the nodal anatomy of a number of species of Piperales and discussed the interrelationships of different genera of the order. Goebel (1931) described the vegetative and flowering stems of *Piper* species and interpreted the stipules as axillary. The present contribution is the continuation of a series that was started sometime ago and deals with the vegetative anatomy of *Piper longum*, *Piper betle* and *Piper subrubrispicum*.

MATERIAL AND METHODS

The materials of *P. betle*, *P. longum*, *P. subrubrispicum* preserved in F.A.A. were kindly supplied respectively by Mr. Y. K. Murty, Mr. S. K. Goswami and Mr. K. M. Vaid. The author's own collection included *P. longum* and *P. subrubrispicum* from Saharanpur and Dehra Dun respectively. Serial transverse and longitudinal sections, 10–12 microns thick, were cut and stained with safranin and fast green in the usual manner.

OBSERVATIONS

External Morphology. While *P. subrubrispicum* and *P. longum* are erect or rambling shrubs *P. betle* is a liana climbing by adventitious roots and thriving well in moist shady localities. This latter species is commonly cultivated in India for its leaves that are chewed. The nodes are conspicuously swollen and the internodes are somewhat cylindrical. In *P. subrubrispicum* the nodes also have a large

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scaly structure, the prophyll, which surrounds the axillary branch and reaches a length of about 4 cm. It is convolute, with a prominent mid-rib which ends in a point far below the apex. The leaves are alternate, simple, cordate palmately veined with a short petiole. The stipules are visible only in *P. betle.*

Vegetative Anatomy. Adult internodes of all the three species studied agree in general and especially in the arrangement and structure of the vascular bundles. A thick cuticle is seen outside the single-layered epidermis. The epidermal cells in *P. subrubrispicum* are unequal in size and those of *P. betle* are papillose. Typical three-celled hydathodes with a balloon-like or disc-like apical cell (*P. betle*) and bicellular (*P. betle*) or multicellular (*P. longum*) trichomes (hairs), are commonly found on the surface.

The cortex in *P. longum* is distinguishable into 2-7 layered collenchyma and several layered parenchyma. Sometimes this parenchymatous region shows lobed outline due to the presence of vascular bundles immediately below. In *P. betle* and *P. subrubrispicum* (Fig. 1) this is distinguishable into three more or less distinct regions : (1) outermost region of about three layers of parenchyma which may also show some stone cells in the latter species : (2) a middle region of 2-3 layers of chlorenchyma with inter-cellular spaces (*P. subrubrispicum*) or 5-6 layers of discontinuous collenchyma (*P. betle*) and (3) an innermost region of 6-9 layers of collenchyma (*P. subrubrispicum*) or 4-6 layers of parenchyma with some chloroplasts (*P. betle*). Secretory cells (oil cells) are also observed in the cortex. No definite pericycle or endodermis could be seen. Chibber (1913) described a definite endodermis in very young stems of *P. betle* and he also interpreted the parenchyma cells below the collenchyma region as pericycle.

There is a very conspicuous mucilage canal in the centre of the internode of *P. subrubrispicum* (Fig. 2) and *P. betle* (Fig. 3) but none in *P. longum* (Fig. 4). In addition to this a ring of smaller canals is also found in *P. betle* separating the two rings of vascular bundles.

The numerous vascular bundles which are collateral and open, are arranged in two (*P. longum*, Fig. 4, and *P. betle*, Fig. 3) or three (*P. subrubrispicum*, Fig. 2) concentric rings. The bundles of the outermost ring are always greater in number than those of the inner, but they are not uniform in their size. This ring has about 15 bundles in *P. longum*, 25 in *P. betle* and more than 35 in *P. subrubrispicum*. The second ring consists of 3-5 bundles in *P. longum* and about 10-12 in *P. betle*. In *P. subrubrispicum* both the second and the third rings show 10-12 bundles each alternating with one another.

It is interesting to note that one of the bundles of the inner ring in *P. betle* is transversely oriented or twisted through 90° (Fig. 3). Chibber (1913) reported several such twisted bundles in the same species.

All the three species agree in the formation of anastomosis and connecting bridges between the vascular bundles of different rings of the internode ; in having 5 or more leaf traces and sympodial growth. However, they differ in details of the nodal structure and hence the three species will be treated separately.

In *P. longum* all the bundles in the sub-nodal region undergo some branching and anastomosing (Fig. 5), those of the two rings fusing freely by connecting bridges and forming a nodal plate or plexus. Five bundles then diverge out from this plexus as leaf traces (Figs. 6-8). Sometimes there may be eight of these but the additional bundles are very small and disappear sooner or later by fusing with the adjacent ones, bringing the number to 5 or 6 at a higher level. With the departure of the leaf traces into the periphery of the cortex the rest of the bundles resolve themselves into two groups. The group adjacent to the leaf traces has many small bundles while the one away from it has a few large ones (Fig. 8). The former supply the axillary branch and the latter the main axis (spike). From the axillary branch some traces move laterally and they constitute the vascular supply to the prophyll and its axillary bud. The prophyll which has about 9 poorly deve-
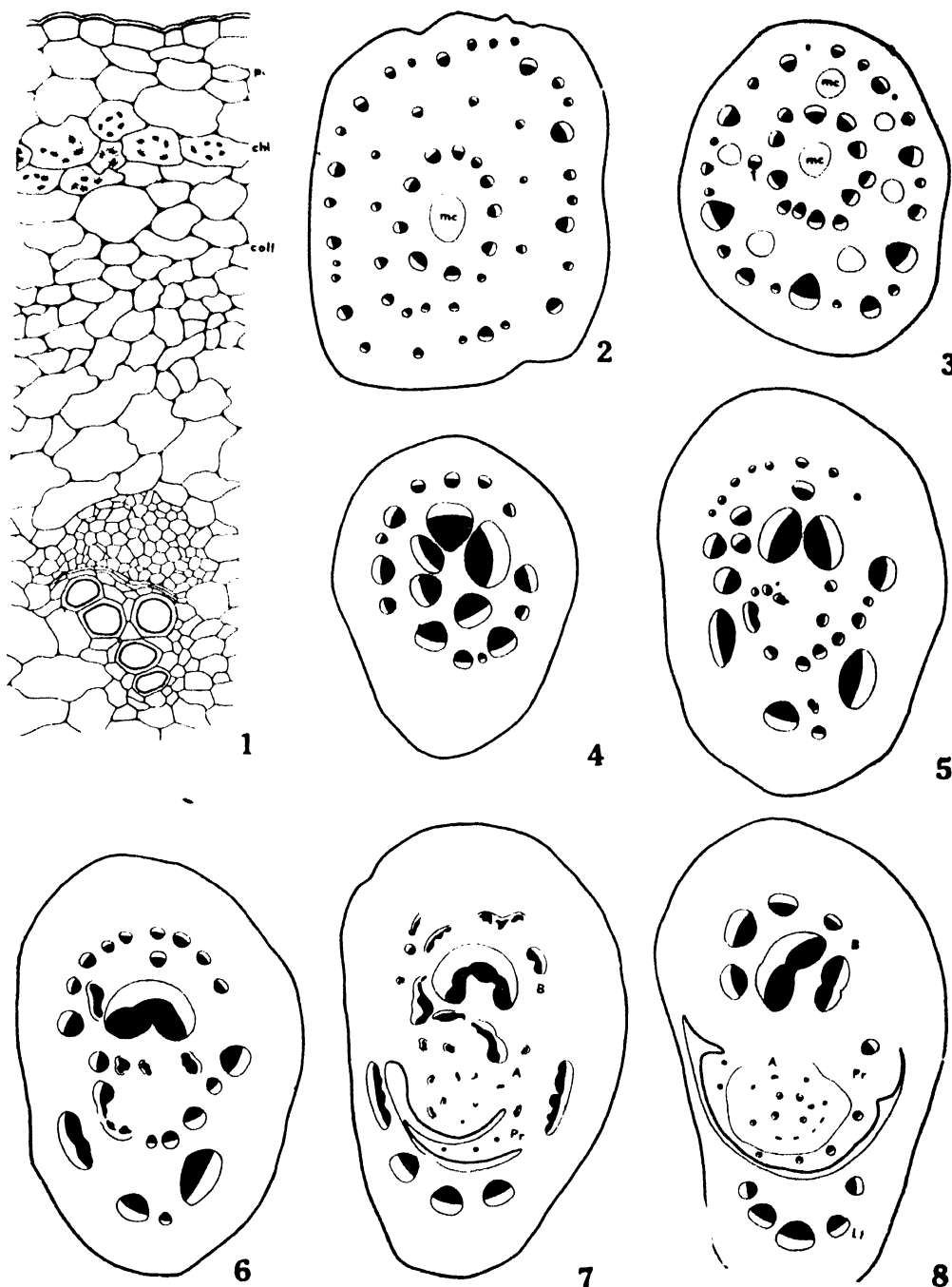
TEXT-FIG. 1.

Fig. 1. A part of a cross-section of internode of *P. subrubrispicum.* × 150.

Figs. 2-4. Respectively cross-section of internodes of *P. subrubrispicum*, *P. betle* and *P. longum.* Note the mucilage canals and inverted bundle in (Fig. 3.T). Figs. 2 & 3 × 12 ; Fig. 4 × 24.

Figs. 5-8. Serial cross-sections of a node of *P. longum* showing the leaf traces and prophyll formation. × 24.
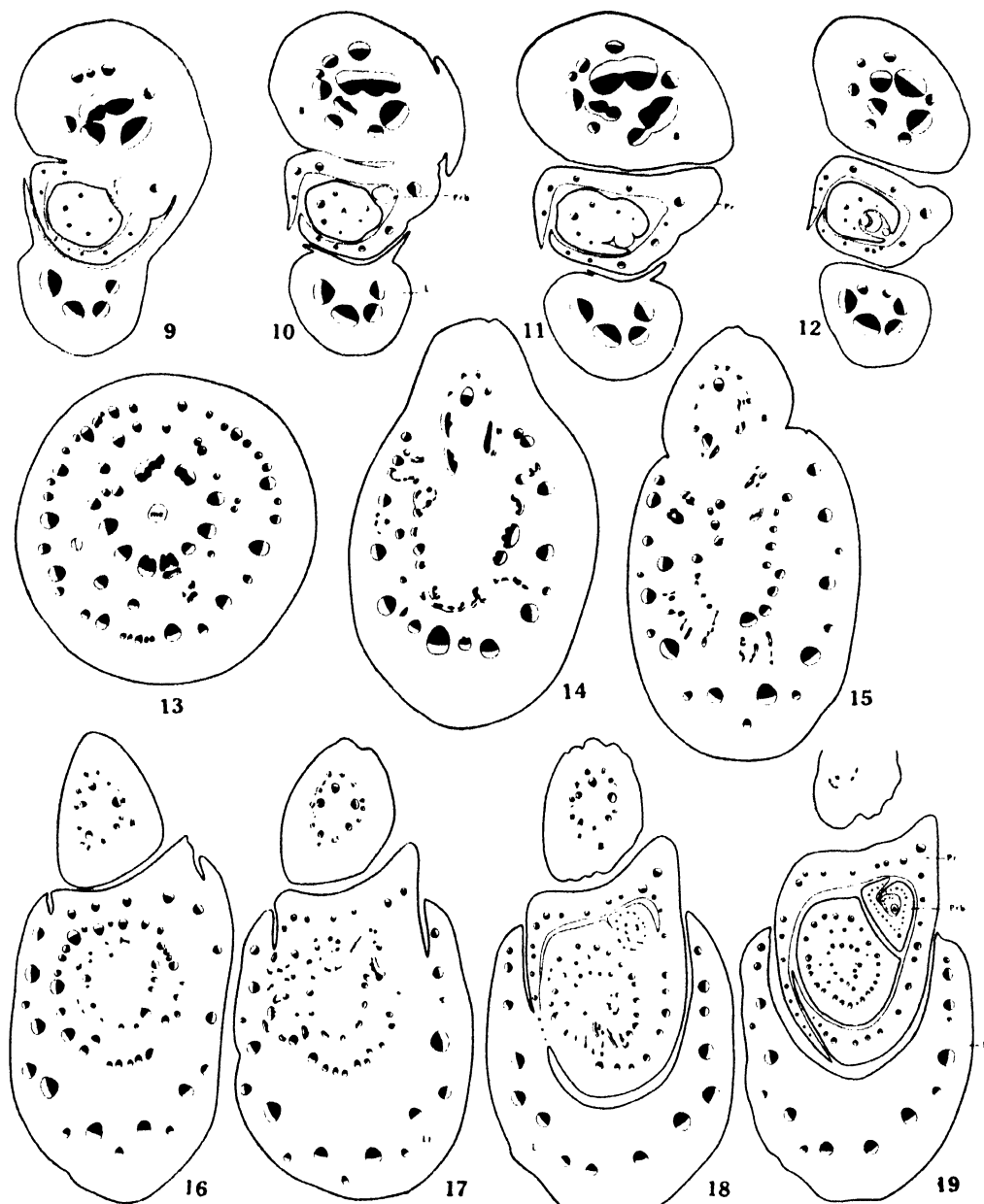
TEXT-FIG. 2.

Figs. 9–12.   Serial transverse sections of a node of *P. longum* showing the separation of
              leaf base, prophyll, prophyll bud, main axis and axillary branch.   × 16.
Figs. 13–19.   Serial transverse sections of a node of *P. subrubrispicum*.   Note the size and
              arrangement of leaf traces.   × 8.

loped bundles, and its rudimentary bud follow the leaf base in their separation
from the node (Figs. 8-12).

In *P. subrubrispicum* the bundles in the sub-nodal region show some enlarge-
ment and then branch and anastomose forming connecting bridges with the outer
ones (Fig. 13).   The central mucilage cavity that runs through the internode

gradually decreases in size and finally disappears at the node being replaced by parenchymatous pith (Fig. 14). Though there appears to be three rings of bundles even at the node they are different from those of the internode. The outermost ring consists of the smaller bundles originally placed in the outer ring of the internode. The second ring contains larger bundles belonging to the outer ring of the internode. The third ring contains the bundles of the second and the third rings of the internode irregularly arranged (Fig. 15).

Simultaneously with this reorganization, a group of bundles appears to move off to one side of the node (Fig. 14). Later on that part of the axis containing this group of two rings of bundles separates as the main axis and then functions as the leaf-opposed spike (Figs. 15, 16).

As the spike is being separated from the node on one side, the leaf traces and the leaf-base are being organised on the other. Most of the bundles of the two outer rings of the node numbering about 20, move out as leaf traces. The rest of the bundles of the node show some branching and reorganise roughly into two rings (Figs. 15, 16). During this process some bundles that constitute supply to the prophyll (Fig. 17) are left in the cortex on the side opposite the leaf base. The leaf base clasps all round the node except on the side where the main axis is cut off. It has alternately large and small bundles, the latter being placed more towards the abaxial side.

With the separation of the leaf base, the prophyll and its bud make their appearance and gradually separate from the axillary branch. The prophyll bud receives several minute traces from the stele of the axillary branch (Figs. 18, 19). The bicarinate prophyll has more than 10 layers of parenchyma in which oil cells are scattered and contains about 12-15 small bundles on either side of the midrib (Fig. 19), one of its margins is overlapped by the other. The axillary branch does not have any central canal at its base but it appears at a slightly higher level.

In *P. betle* the bundles in the sub-nodal region enlarge somewhat in size and become surrounded by a fibrous sheath. On approaching the node those of the outer ring branch and anastomose (Figs. 20-22). They develop connecting bridges between themselves and between those of the inner ring and send off seven large alternating with four small leaf traces into the cortex (Figs. 23-25). In addition to these 11 traces there pass out, into the scarious margins of the leaf base, two very small traces one on each side (Fig. 23, St).

The twisted bundle (Fig. 20) that is so constantly seen in the internodal region appears to become normally oriented and merges with the bundles of the inner ring. It is thus not visible in the nodal region. The central mucilage canal becomes very much elongated transversely and the peripheral ones merge into larger groups (Figs. 21, 22). Just above the separation of the leaf from the node the central cavity again becomes very small but it never disappears completely (Fig. 25).

Some bundles on the opposite side of the leaf diverge out a little but soon disappear without separating into a distinct structure (Fig. 23, B). In all probability this is the abbreviated continuation of the main shoot. The growth of the axis thus appears to become sympodial. With the separation of leaf base, the axillary branch also shows its first leaf, the prophyll, and sends off some very rudimentary traces for the prophyll and its axillary branch (Fig. 25, Pr.).

The structure of the petiole is almost similar in all the three species studied except for minor differences. It is more stout in *P. betle* and *P. subrubrispicum*. It is generally plano-convex in corss-section with a central groove on the adaxial side which varies in depth in different regions of the petiole of different species.

The petiole has a single layer of epidermis, the cells of which are slightly papillose in *P. betle*. Hydathodes are more common in *P. betle*. Just below the epidermis there is a discontinuous layer of stone cells in *P. subrubrispicum*. Collenchyma is well developed in discontinuous patches in *P. subrubrispicum* and in *P.*
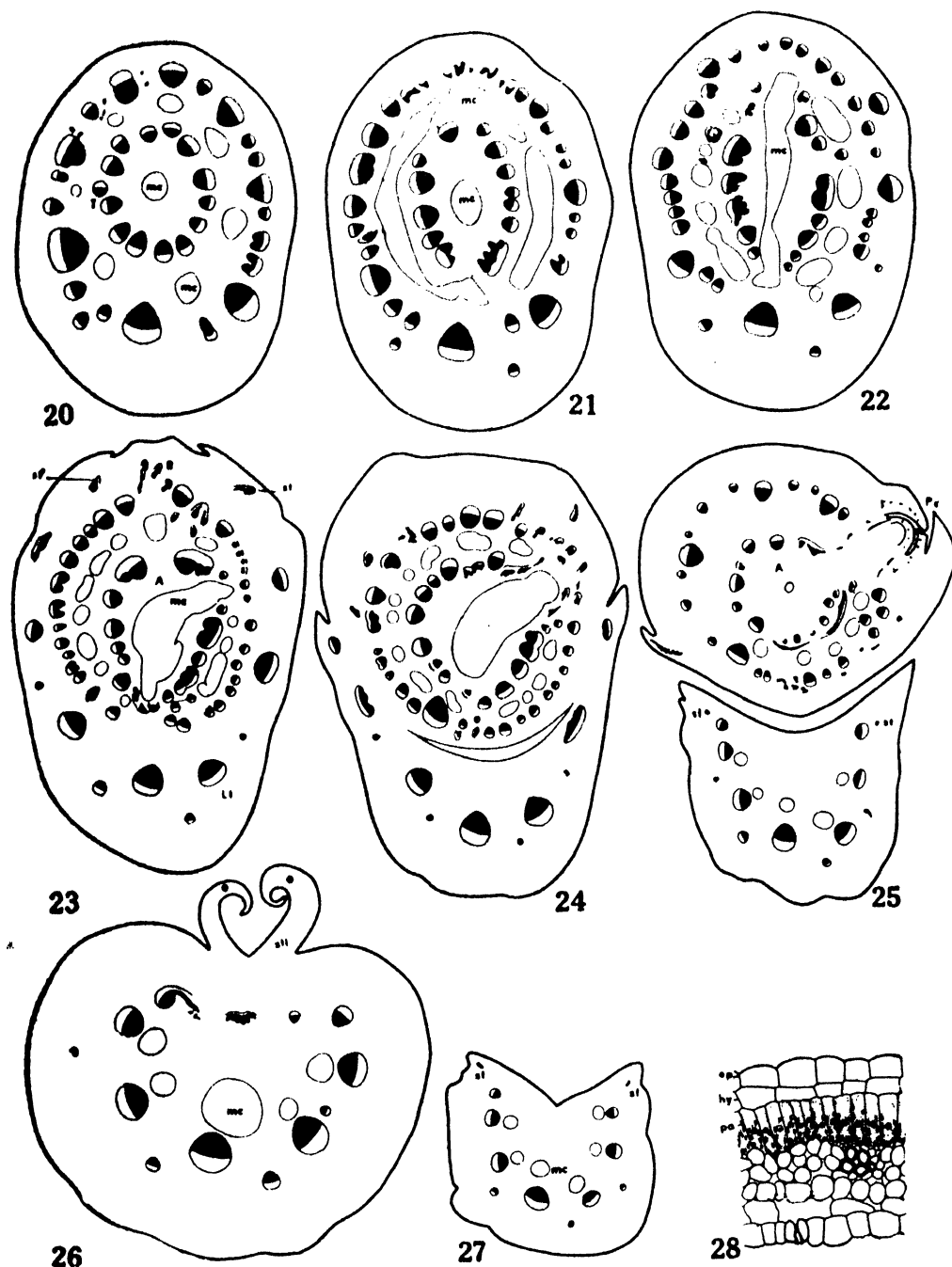
TEXT-FIG. 3.

Figs. 20-25.   Serial transverse sections of a node of *P. betle*.   Note mucilage canals and stipula
                        traces.   × 10.
Fig. 26.   Cross-section of a petiole of *P. betle* showing the stipule.   × 20.
Fig. 27.   Cross-section of a petiole of *P. betle* showing the stipular traces.   × 13.
Fig. 28.   A part of transverse section of a leaf of *P. longum*.   × 229.

betle those below the upper epidermis are typically tabular resembling in their arrangement cork cells. Secretory cells and calcium oxalate crystals are found scattered in the parenchyma region. The vascular bundles of the petiole resemble those of the internode but they have poorly developed cambium. Chibber (1913) suggested the bundles in the petiole of *P. betle* to be schizostelic. Their number varies somewhat. There may be 4-5 bundles of almost of the same size and occurring in open arc (*P. longum*, Figs. 10, 11) ; seven large alternating with four small bundles in addition to the two minute stipular ones (*P. betle*) ; and 9 large alternating with 8 small bundles (*P. subrubrispicum*).

The vascular bundles especially those at the margins of the open arc show branching and fusion bringing about variation in their arrangement and number (Fig. 26). This generally results in their arrangement in a ring (Figs. 12 and 27). These extra bundles are always small and vary from 2—6. In *P. betle* a mucilage canal is seen inner to each of the larger bundles at the base of the petiole (Fig. 27).

The lamina is palmately and prominently 5- many veined. Some collenchyma is seen in the midrib region and below the veins also. The epidermis is thick-walled with a cuticle. The palisade is one (*P. longum*, Fig. 28) or two (*P. subrubrispicum*) layered, followed by 3-4 layers of spongy parenchyma. Secretory cells are confined to this region. Stomata are on the abaxial surface and their guard-cells show upper ledges. The vascular bundles resemble those of the petiole.

DISCUSSION AND CONCLUSIONS

Anatomy of the adult internode does not warrant any detailed comments. Variations in the number and arrangement of bundles in the internode, and in the behaviour of the mucilage canal when present, appear to be distinctive of different species and these have been considered by Solereder (1908) and Metcalfe and Chalk (1950), as of sufficient taxonomic value in delimiting the species of *Piper*.

The structure of the node also is somewhat distinctive of different species and can be determined by the number of leaf traces, mucilage canals etc. As a rule, each node bears a leaf, a leaf-opposed spike, and a bud which carries on further vegetative growth. The latter in turn bears a prophyll which has a bud in its axil, almost at the level of the node of the parent axis. The relationships of these different organs at the node though not externally visible are very clearly established by the course and behaviour of their vascular bundles. There is little doubt that the leaf-opposed spike which has a cylinder with fewer vascular bundles is the continuation of the main axis which has limited growth. The branch in between this and the leaf is naturally the axillary branch which carries the sympodial growth further. Its first leaf is a prophyll which bears a bud in its axil.

Chibber (1913) offered a different interpretation of the node in *P. betle*. He considered the prophyll as a scale leaf or sheath borne on the abbreviated internode and the normal leaf borne on an elongated internode. He, therefore, assumed that in this case the branch consists of abbreviated internodes, alternating with elongated internodes. Such an interpretation is obviously incorrect and is due perhaps to a misinterpretation of the prophyll which is borne not on the main axis but on the axillary branch.

There has been some difference of opinion as to whether stipules occur in all species of *Piper* or not. There is little doubt that they exist in *Piper betle* where each stipule receives a trace that immediately branches.

Though no stipules or vascular supply is evident in *P. longum* a structure similar to the one found in *P. betle* is observed half detached on the upper surface of the petiole. Whether it is a rudimentary, reduced axillary stipule or simply a thin wing of the petiole is, however, difficult to say.

In *P. subrubrispicum* there is no evidence whatsoever of the presence of stipules. But in the arrangement of bundles in the leaf base it agrees with that of *P.*

tiliaefolium which has been interpreted by Rousseau (1927) to be stipules. Out of more than fifteen bundles that occur in the leaf base of *P. subrubrispicum* the smaller ones are placed alternately with the bigger ones more towards the abaxial side. However, no alternation was seen in the last three bundles in the margins of the leaf base. Such an arrangement of bundles in a single file led Rousseau (1927) to interpret the margins of the leaf base in *P. tiliaefolium* as stipular in nature. If that is so then *P. subrubrispicum* also has stipules though it is doubtful if this much of anatomical evidence is quite adequate to interpret the margins as stipular.

Prophyll or the first leaf of the axillary branch has been described in the majority of angiosperms. Dicotyledons have been regarded as characterised by two, and Monocotyledons by one prophyll. But many exceptions to this rule are already on record (Arber 1925 ; Foster 1932). Blaser (1944) discussing the morphology of Cyperaceae reviewed the previous work, the meaning, nature and variation etc., in the prophyll of angiosperms and came to the conclusion that it is a leaf or leaf-like structure and not a distinct morphological entity.

Like many other exceptions *Piper*, too, like *Peperomia* (Murty, 1958), has a single prophyll. Foster (1932) considered the single bicarinate prophyll in *Carya* as double on the basis of vasculature and presence of two buds. Similarly Arber (1925) interpreted some of the prophylls of Monocots to be double. But in *Piper* it is really a single structure as it has a single mid-vein and one axillary bud. In the past prophyll has been equated with parts of a fully developed leaf (De Candolle 1866 ; Rousseau 1927). This is apparently a wrong approach.

ACKNOWLEDGEMENTS

REFERENCES

Arber, A. (1925). Monocotyledons. Cambridge.
Blaser, H. W. (1944). Studies in the morphology of the Cyperaceae. II. The prophyll. *Amer. Bot.*, **31**, 53-64.
Chibber, H. M. (1913). The morphology and histology of *Piper betel* Linn. (the Betel-vine). *Linn. Soc.*, **41**, 357–383.
De Candolle, C. (1866). Memoire sur la Familie des Piperacees. *Mem. Soc. Physi et Histor. Natural de Geneve*, **18**, 1–32.
Foster, A. S. (1932). Investigations on the morphology and comparative history of development of foliar organs. IV. The prophyll of *Carya Bucklevi* var. *Arkensana*. *Amer. Jour. Bot.*, **19**, 710–728.
Goebel, K. (1931). Blütenbildung und sprosegestaltung. (Anthokldien und Inflorescenzen). Jena.
Metcalfe, C. R. and Chalk, L. (1950). Anatomy of the Dicotyledons. Vol. II, London.
Murty, Y. S. (1958). Studies in the order Piperales. 1. A contribution to the study of vegetative anatomy of some species of *Peperomia*. *Phytomorph*. (in press).
Rousseau, D. (1927). Contribution a l' anatomie comparete des Piperacees. *Acad. Roy. Belgique Cl. Sci. Mem.*, **9**, 3–45.
Solereder, H. (1908). Systematic Anatomy of the Dicotyledons. Vol. II. Oxford. (Trans. L. A. Boodle, and F. E. Fritsch).

LEGEND

A—axillary bud ; B—main branch ; Chl—chlorenchyma ; Coll—collenchyma ; leaf base ; Lt—leaf traces ; mc—mucilage canal ; P—petiole ; Pa—parenchyma ; Pal—palisade Pr—prophyll ; Prb—prophyll bud ; St—stipular trace ; Sti—stipule ; T—inverted bundle.

A STUDY OF NITROGEN FIXATION IN THE DETACHED ROOT NODULES OF SUNNHEMP

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and
Bimla Budhraja, *Government College for Women, Ludhiana*

Abstract

The root nodules attached to the small pieces of roots absorb nitrogen at a very high rate, when compared to those detached from the roots.

The attached root nodules show marked increase in total nitrogen and amide nitrogen, which is not noticed in the case of nodules detached from the roots.

Nitrogen can be fixed only by the nodules attached to small pieces of root.

It is common knowledge that leguminous plants have the power to fix atmospheric nitrogen through the agency of symbiotic bacteria in the root nodules. This was noticed, as early as 1853, by Ville (1853-1857) and confirmed by Hellriegel and Wilfarth (1888). In this investigation the influence of detachment of the root nodules from the host, on their nitrogen fixation, was studied under anaerobic conditions.

Experimental procedure

Randomized samples of two months old plants of Sunnhemp, brought from the field immediately before the start of the experiment, served as the source of material. The root nodules of equal size were used. Nodules were detached in distilled water by the help of a pair of scissors, taking care not to take any root tissue with them. A study of nitrogen absorption was carried out with Warburg manometric technique. The analysis of nitrogen fractions was carried out by the micro-kjeldal apparatus on fresh weight basis. Two sets of experiments were conducted.

1. With nodules detached from the roots.
2. With nodules attached to small pieces of roots.

Results

Nitrogen Absorption :

Fig. 1 represents the nitrogen absorption of detached and attached root nodules. A careful study of the nitrogen absorption curve, in the case of detached root nodules, shows that abosrption starts at a high level (0.8 μ), but falls gradually to zero after 60 minutes. No absorption is observed in the next 10 minutes. Thereafter, a negligible amount of gas is absorbed, which comes to a standstill after the nodules have been in nitrogen for 120 minutes.

The rate of absorption in the case of attached root nodules starts with a very high value (1.9 μ) and remains unsteady, yet high, for fifty minutes. Then the absorption rate follows a uniform course but at a very high level.

TEXT-FIG. 1.
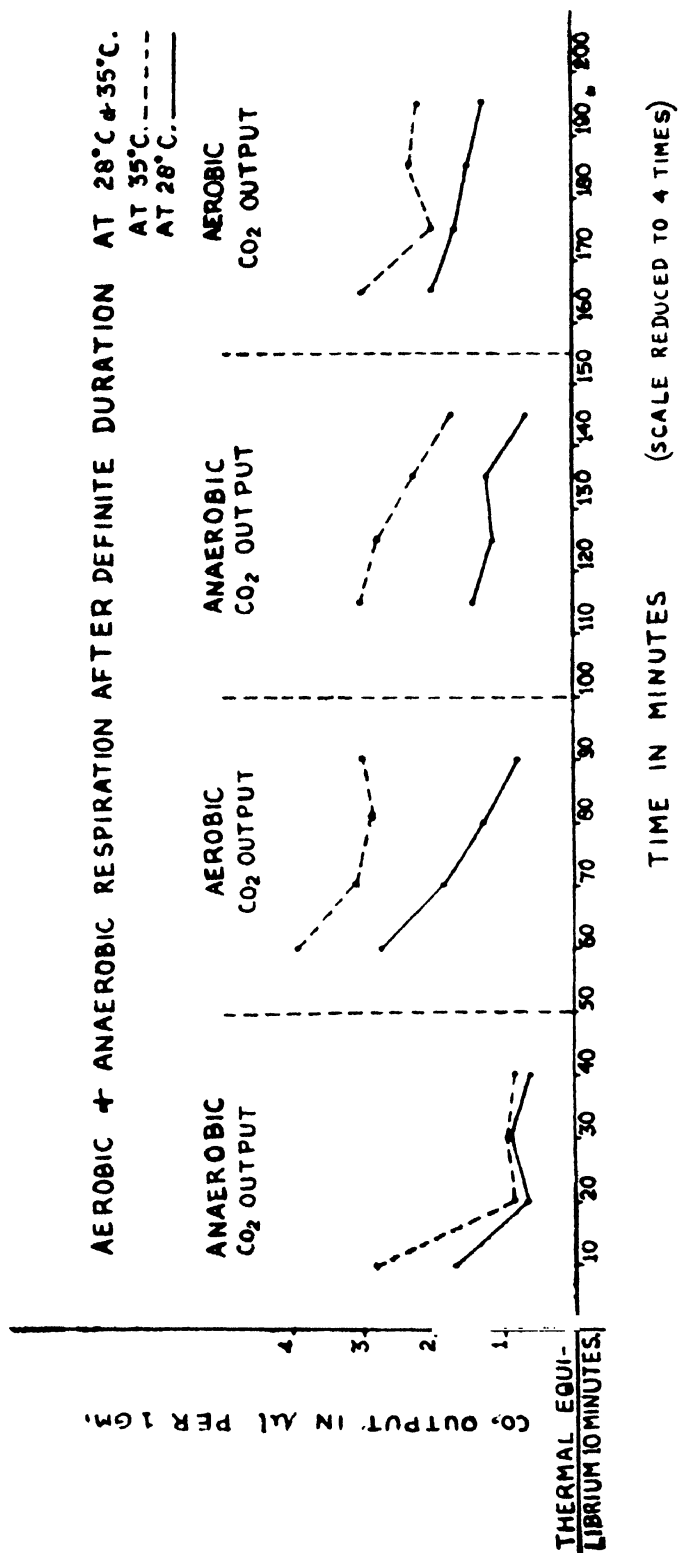
Chemical Analysis : Total Nitrogen :

The analysis of total nitrogen from detached root nodules after anaerobiosis in nitrogen atmosphere, does not show any increase in total nitrogen, irrespective of the duration in nitrogen atmosphere.

The nodules attached with small pieces of roots (Fig. 2) when kept in nitrogen atmosphere and analysed for total nitrogen (only the nodules taken for analysis) show a totally opposite result. In this case a large amount of increase is observed over the control. This increase in total nitrogen increases with the increase in the period of anaerobiosis, reaching its maximum after 20 hours. A further increase in the period of anaerobiosis does not exhibit any increase in total nitrogen.

ALCOHOL FORMATION

IN CONTROL ▬

AFTER 1 HR. ANAEROBIOSIS ▯

,,   3 HRS   ,,

EXPOSURE TO AIR FOR ½ HR. AFTER 3 HRS. ANAEROBIOSIS

EXPOSURE TO AIR FOR 1 HR. AFTER 3 HRS. ANAEROBIOSIS ▬

PERCENTAGE OF ALCOHOL

3·0%
2·5%
2·0%
1·5%
1·0%
0·5%
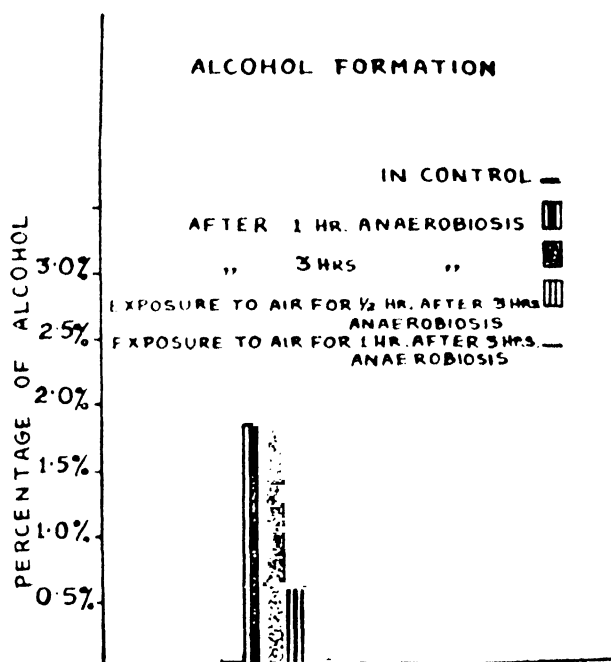
TEXT-FIG. 2.

No increase in total nitrogen was observed in hydrogen.

Amide Nitrogen :

Amide nitrogen (Fig. 3) also increases when the root nodules attached to small pieces of roots are kept in nitrogen atmosphere. The amides increase with the increase in the period of anaerobiosis upto 20 hours. Thereafter no increase in amides is noticed.

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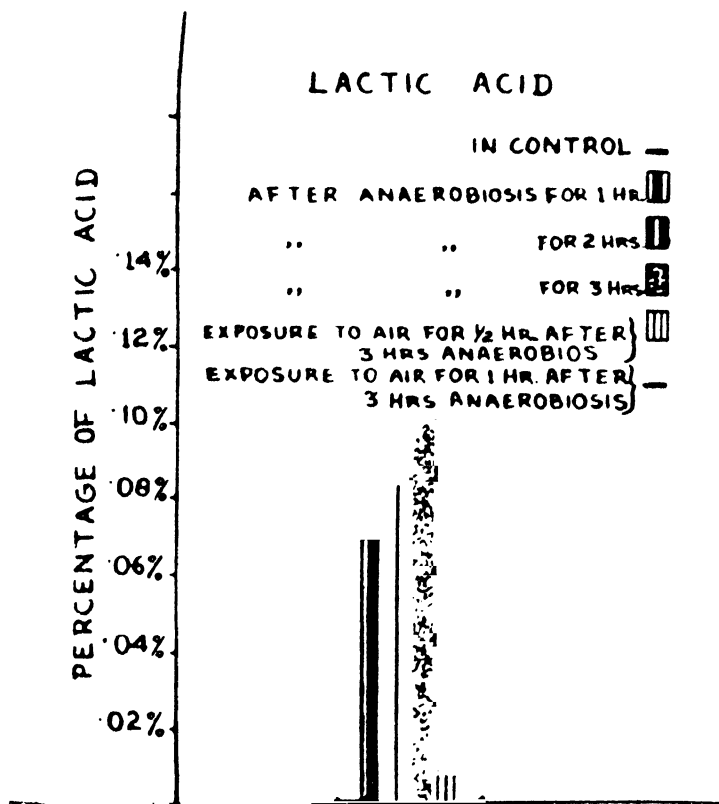
TEXT-FIG. 3.

The hydrogen atmosphere seems to have no effect on the amides of root nodules detached from the roots, or attached with small pieces of roots.

DISCUSSION

When nitrogen absorption curves in both the cases are compared, it is found that the rate of nitrogen absorption in the case of detached nodules can be taken as negligible as compared to the rates of absorption in attached nodules. Uptake of nitrogen in the case of detached ones is low in the beginning and gradually falls to zero. On the other hand, in the case of attached root nodules the consumption of nitrogen is quite different. Here continued absorption of nitrogen is noticed. The latter is comparatively low (though much higher than that observed in the case of detached root nodules) in the beginning of the experiment, but increases after fifty minutes and continues at the same rate for a considerable time. The slight absorption of nitrogen in the excised nodules can, therefore, be attributed to its solubility in the nodule sap, which may not be fixed by nodules at all.

The chemical analysis of total nitrogen and amide nitrogen reveal that the nodules, attached with small pieces of roots. can fix nitrogen in nitrogen atmosphere. The maximum period of fixation is 20 hours, beyond which no increase in the uptake

of nitrogen is noticed. This could probably be due to the curtailment of the translocation of the end products of fixation, resulting in the retardation of the process.

There was no increase in the amide or total nitrogen in hydrogen atmosphere.

The detached root nodules did not show any increase. This would point to the possibility that the available carbohydrate necessary for the fixation of nitrogen present in the nodules at the time of the detachment might have been exhausted during respiration. The plant does not show any decrease in nitrogen; but were the experiments to be conducted for a longer period a decrease in the nitrogenous compounds is likely to be detected owing to their formation as respirable substrates. This work is at variance with Bond (1957) where he found that the excised nodules of casuarina fix nitrogen for a few hours.

REFERENCES

Bond. G. (1957). Isotopic studies of N_2 fixation in nonleguminous root nodules. *Annals of Bot.* **21**.

Hellriegel and Wilfarth (1888). Untersuchungen über die stickstoffnahrung der Gramineen und Legumino-sen, Beilage Z. Rüben zucker-Ind-Reich.

Ville (1853-58). Recherches expérimentales sur la végétation. Paris.

METABOLIC CHANGES IN POTATO TUBERS UNDER ANAEROBIOSIS

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and

BIMLA BUDHRAJA, *Government College for Women, Ludhiana*

ABSTRACT

When aerobiosis is followed by anaerobiosis the rate of CO_2 output rises abruptly and then slowly falls to a lower level.

Alcohol is formed in the tissues during first hour of anaerobiosis, but does not increase with duration of anaerobiosis. Lactic acid accumulates when potato tissues are kept in nitrogen.

Accumulation of lactic acid may be inhibiting the output of carbon dioxide under anaerobic conditions.

Plant tissues brought into air after a brief period of anaerobiosis, show a rate of CO_2 output which is far higher than that in air line (Parija 1928, Barker and Saifi 1952). In the present investigation an attempt has been made to compare the catabolic changes in the potato tissues, brought to air after a period of anaerobiosis, with those in presence of air.

MATERIAL AND METHODS

Respiration of potato tubers (Var. *phulwa*), in presence of air and without, was determined by Warburg manometric technique. Discs of 3 mm. thickness and 1 cm. dia. were cut from the tubers, from non-bud area, by means of a corkborer and hand microtome. Five discs were taken in each chamber in distilled water.

The respiration rates of potato tissue kept under anaerobic conditions followed by aerobiosis were studied at 28°C and 35°C. The tissue was kept in nitrogen for 40 minutes, and the gas was then flushed out with air and the respiration rate in air was determined for 40 minutes. The process was repeated again.

Estimations of Lactic acid were carried on by Smith and Coma's method modified by Barker and Saifi (1952).

For alcohol estimations, the potato tissue was boiled for 10 minutes, crushed and then distilled.

10 c.c. each of standard solution of alcohol viz., 0.05 per cent, 0.1 per cent, 0.15 per cent. 0.2 per cent and 0.25 per cent were taken in pyrex test tubes. To each tube 1 c.c. of 2 per cent potassium dichromate was added and solutions acidified with a few drops of sulphuric acid. The tubes were then shaken well and sealed.

10 c.c. of the potato extract, was taken in a test tube and the reagents were added as described above. The solutions were then compared with the standard solutions with the help of photo-electric colorimeter.

Analysis was carried on fresh weight basis.

RESULTS

Fig. 1 shows the rate of respiration of potato discs alternately placed in anaerobic and aerobic conditions at 28°C and 35°C. It is clear from the figure that the rate of CO_2 output in nitrogen atmosphere is very high in the beginning, but falls
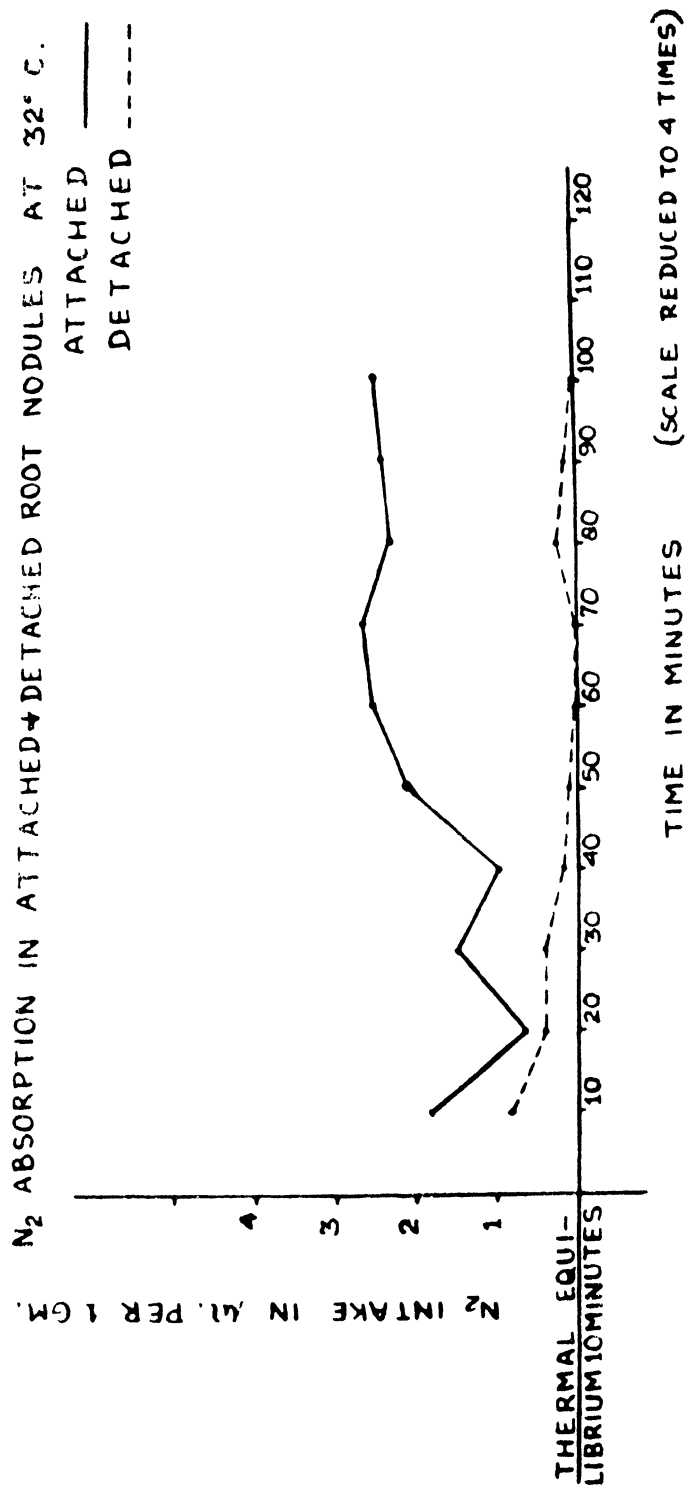
TEXT-FIG. 1

to a very low level after ten minutes and then follows a course at the same level at both temperatures. The same tissue when exposed to air, shows a sudden rise in the rate of CO_2 output. The repetition of the same fact is observed when the potato discs are placed under anaerobic conditions once again and brought back to air.

ALCOHOL

The results of alcohol estimations are depicted in Fig. 2 which shows that no alcohol is found in control potato tissues and those which were kept in nitrogen

NITROGEN FIXATION IN ROOT NODULES
OF SUNHEMP IN NITROGEN
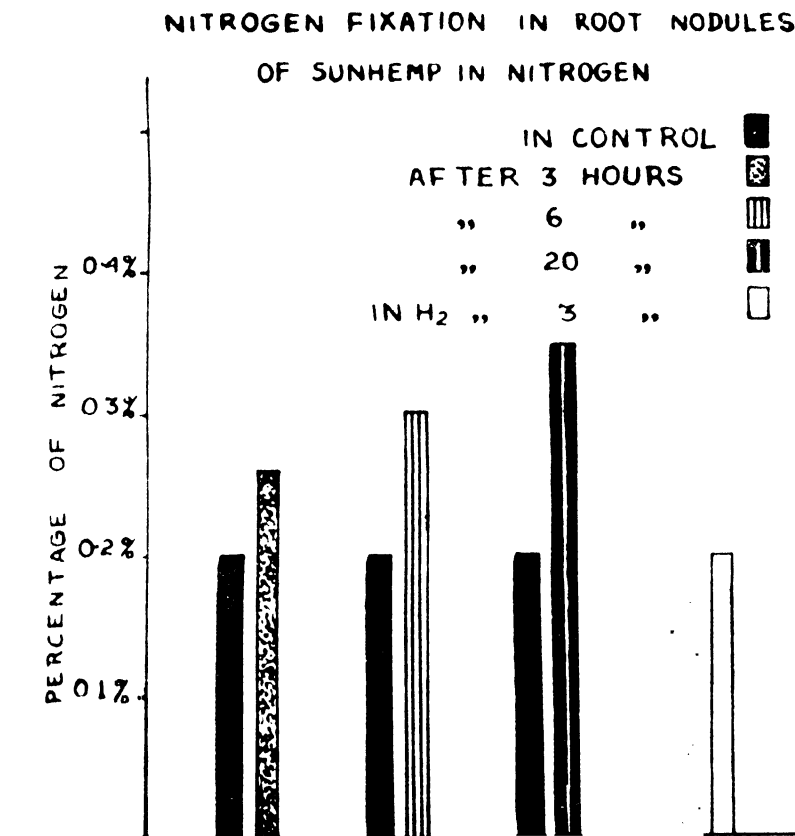
TEXT-FIG. 2.

for three hours and then exposed to air for 1 hour ; while the potato discs kept in nitrogen for 1 and 3 hours, revealed 0.157 per cent of alcohol. Potato discs kept in nitrogen for 3 hours and then exposed to air for 30 minutes showed 0.057 per cent alcohol.

LACTIC ACID

The highest amount of lactic acid is recorded in the tissues kept in nitrogen for 3 hours (Fig. 3). This is followed by the sets exposed to nitrogen for 1 and 2

hours. The set which was kept in nitrogen for 3 hours and then exposed to air for $\frac{1}{2}$ hour showed the least amount of lactic acid, while the control and the tissue

AMIDE NITROGEN INCREASE IN
ROOT NODULES OF SUNHEMP IN
ANAEROBIC CONDITIONS
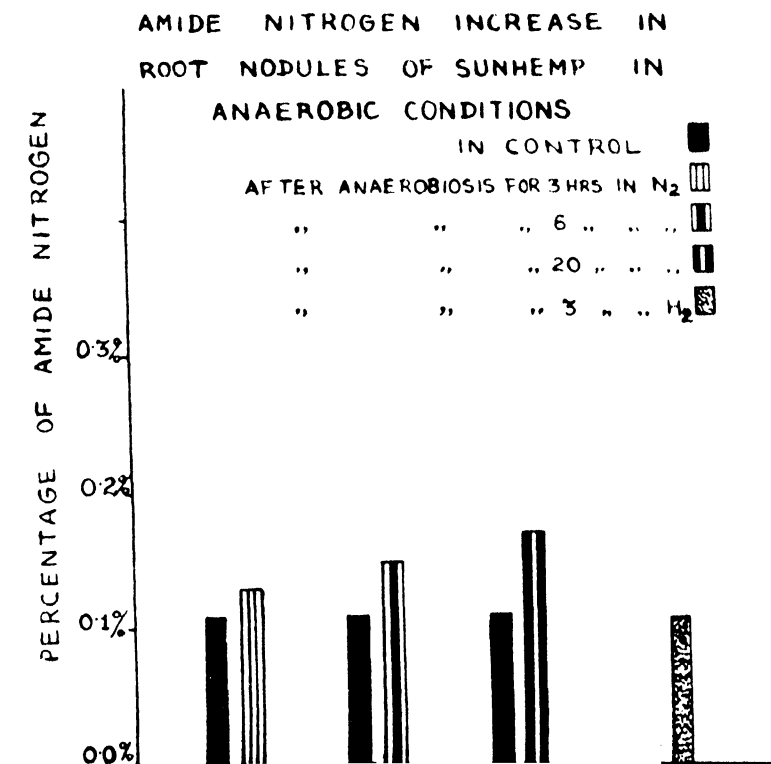
TEXT-FIG. 3.

kept in nitrogen for 3 hours and then exposed to air for an hour, were totally devoid of lactic acid.

DISCUSSION

It is well established that the catabolism of carbohydrates in presence of air involves an initial break-down to pyruvic acid, which is followed by oxidation of these products ; and under anaerobic conditions transformation of pyruvic acid to CO_2 and alcohol. Barker and Saifi (1952) from their experiments on potato tissue were able to conclude that under anaerobic conditions pyruvic acid is reduced to lactic acid and alcohol is formed only if the tubers were cut or otherwise injured.

In the present experiments, lactic acid was found to increase with the period of experimentation but the amount of alcohol was at a constant level.

CO_2 output always showed a rapid fall on being transferred to anaerobic conditions which remained at a low level throughout the period of anaerobiosis. When the tissue was brought back to air it recorded a steep increase. This (pastcur effect) was observed by many authors (Parija 1928, Barker and Saifi 1952).

Since the experiments also show the increased production of CO_2 in air after anaerobiosis, it is clear that there is a definite conservation of carbon in aerobic

conditions and oxidation of the accumulated products (lactic acid). The chemical analysis of the tissues before and after the treatment of anaerobiosis shows that the anaerobic respiration is not identical to alcoholic fermentation, but resulted in the formation and then accumulation of lactic acid (Fig. 3). Formation of a little quantity of alcohol, and accumulation of large quantities of lactic acid explains the evolution of the low amounts of CO_2 on exclusion of air. The high rate of CO_2 production by tissue in air after anaerobiosis also indicates that the accumulated lactic acid probably has been oxidized to pyruvic acid and through Kreb's cycle to CO_2 and H_2O.

ACKNOWLEDGEMENT

REFERENCES

Parija, P. (1928). Analytical studies on plant respiration. *Proc. Roy. Soc.* London, **103**, **B**, 446-90.

Barker, J. and Saifi, A.F. el (1952). Experimental studies of the formation of CO_2, Lactic acids and other products in potato tubers under anaerobic conditons. *Proc. Roy Soc. London*, **140**, **B**, 508-555.

OBSERVATIONS ON INCREASED PERMEABILITY DURING HYPOTHERMIA AND ITS EFFECT ON PLASMA PROTEINS

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ABSTRACT

The change of permeability during hypothermia was investigated after injecting xylose and inulin intravenously. The arterio-venous differences of these nonutilisable carbohydrates were compared in isothermic and hypothermic limbs. It was found that these substances diffused out of blood vessels and attained equilibrium with the extravascular compartment, at a quicker rate, during hypothermia. In spite of considerable decrease of plasma volume during hypothermia, the total plasma protein was diminished, indicating considerable protein loss from vascular compartment. Due to lower molecular weight the loss of albumin was relatively greater than other plasma proteins.

INTRODUCTION

Investigations on the utilization of carbohydrate during hypothermia brought to light several facts (Sen *et al.*, 1957). It was observed that the arterio-venous difference of glucose in blood was increased during the initial stage of hypothermia, induced either by extracorporeal method or by surface cooling. During the sugar tolerance test, the pattern of arterio-venous difference in the hypothermic limb showed some departure from the normal arterio-venous difference observed in the isothermic limb of the same animal. Relatively quicker removal of arterial blood sugar at the initial stage of hypothermia and slower removal at a later stage at the identical sugar level suggested an increased permeability of the capillaries to blood sugar. As glucose is an utilizable sugar, its equilibrium in the tissue was influenced by a dynamic state of metabolic activity. Stimulation of heat production due to cold may considerably influence such event (Brody, 1945). A better interpretation could, therefore, be obtained by studying the arterio-venous difference in hypothermia, with inert and non-utilizable sugars like xylose and inulin. Increased capillary permeability is also a likely factor to alter the protein components of blood plasma, both quantitatively and qualitatively. The total plasma protein and the different components were, therefore, studied both in hypothermic and normothermic blood.

EXPERIMENTS

Cats weighing from 2-3 kg. were used in the experiments. They were anesthetised with intramuscular injection of urethane (2 gm/kg.). To determine the change of permeability towards inert sugars like xylose and inulin, one of the hind limbs of the urethanised cat was brought to the desired hypothermic condition by ice packing, while the other was gently warmed to maintain the isothermic temperature. The temperature of the deeper tissues was determined directly

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with a centigrade thermometer.   When the desired experimental thermal condition was attained, 40 per cent solution of xylose containing 0.5 gm/kg. body weight was intravenously injected in the femoral vein of the cats.   In another series o- experiments, different amounts of inulin were injected intravenously.   The arteriof venous differences of these sugars in the hypothermic and isothermic limbs were determined by estimating xylose (Drury, 1948) or inulin (Little, 1949) in arterial and venous blood samples at different intervals (Tables I and II).   Both cats and dogs were used for the study of plasma protein during hypothermia.   2 gm/kg. urethane was used intramuscularly for anesthetising the animals.   Desired degree of hypothermia was produced either by surface cooling or by extracorporeal method (Mukherjee *et al.*, 1956).   Blood serum and heparinised blood plasma, prepared from the arterial blood of animals in hypothermic and prehypothermic condition were analysed for total protein (Hawk *et al.*, 1954).   Different fractions of plasma proteins were determined by paper electrophoresis carried out with barbiturate buffer (*p*H 8.6 ionic strength 0.05) in 5 hours run at 8 mA current.   Densitometric evaluation was carried on bromophenol blue stained electrophorogram (Table III).

RESULTS AND DISCUSSION

Both xylose and inulin are non-utilizable sugars and are permeable to the capillary walls.   Inulin being of higher molecular weight diffuses out at a lower rate than xylose and its distribution is limited to a more restricted space.   Like other unutilizable sugars, their rate of diffusion is governed by simple physical laws of permeability.   Xylose being of lower molecular weight diffuses out of blood vessels at a quicker rate than inulin.   For this reason the arterio-venous differences of xylose concentration are more remarkable in the hypothermic and isothermic limbs in comparison with similar observation in respect to inulin, recorded at 5 minutes.   At this stage, the removal of these saccharides from the arterial blood was greater in the hypothermic limb.   In the later stage of experiment, however, the removal of sugar became slower in the hypothermic limbs, and in some instances there were additions to the venous blood instead of removal.   The above obser- vations suggest a quicker attainment of equilibrium in the hypothermic limbs, when the concentration of saccharides was high in the arterial blood.   This produced higher extravascular concentration of xylose and inulin in the hypothermic limbs. Therefore, at the later stage of experiment the diffusion gradient in the hypothermic limbs was more unfavourable for the saccharides to pass out of artery to the extra- vascular space.   These data corroborate similar observations with glucose (Sen *et al.*, 1957).

Altered capillary permeability produces changes in the concentration of plasma proteins.   Plasma and serum proteins always decreased in the hypothermic animals.   In the ten cases studied decrease in serum protein ranged from 3.7 to 17.7 per cent.   Albumin, the plasma protein with lowest molecular weight, decreased in every case, the percentage of decrease in albumin was greater than that of total protein in all cases except in cases 1 and 8.   In most cases the globulin fraction also decreased to slight extent, but the percentage of decrease was consider- ably lower than the albumin fraction of the blood plasma.   This is consistent with higher molecular weight of plasma globulins.   It has been reported that in hypo- thermia there is haemoconcentration, plasma volume being reduced to a greater extent than the total blood volumes (Mukherjee *et al.*, 1957).   So, even in cases where albumin and globulin concentration remained the same in normothermic and hypothermic serum samples, it meant some loss of albumin and globulin from the serum.   The decrease in total protein in all cases and decrease in albumin/globulin ratio in 8 out of 10 experiments strongly suggest that an increase in permeability of blood vessels to serum proteins is responsible for serum protein depletion in hypothermia.

When relative change in concentration of individual globulin components of blood during hypothermia was similarly assessed, the results were not so consistent as to justify such sweeping generalisation as to indicate permeability being the only reason for all changes in plasma proteins. So many homeostatic mechanisms are at play during the induction of hypothermia, that their total contribution may not be very insignificant at a particular moment. Cold stress for instance, liberates cortical hormones in the circulation. Some components of the plasma globulin may be electrophoretically altered by binding a part of the cortical hormone (Sandberg, 1957). A thyroxine binding specific component is also present in the globulin fraction (Robbin and Roll, 1957). This may form a complex with the increased amount of thyroxine secreted during application of cold. Slight change in the electrophoretic pattern may be due to addition of tissue components and complex formation. The increase in albumin/globulin ratio in cases Nos. 1 and 8 suggests that removal of globulin by mechanism, such as deposition, may also be responsible for complicating the globulin picture.

TABLE I

Arterio-venous difference of intravenously injected xylose (0.5 gm./kg. body weight) in the isothermic and hypothermic limbs of urethenised cats.

Cat No.	Minutes after injection	Gamma of Xylose/ml. of blood			Arterio-venous difference in	
		Artery	Isothermic vein	Hypothermic vein	Isothermic limb	Hypothermic limb
1	5 min.	770(36°C)	720(36°C)	510(28°C)	—50	—260
	20 min.	390(36°C)	330(36°C)	367(28°C)	—60	23
2	5 min.	503(37°C)	431(37°C)	300(27°C)	—72	—203
	20 min.	266(37°C)	240(37°C)	200(27°C)	—26	—66
3	5 min.	740(38°C)	680(38°C)	599(28°C)	—60	—142
	20 min.	456(38°C)	399(38°C)	428(28°C)	—57	—28

TABLE II

Arterio-venous difference of intravenously injected inulin in isothermic and hypothermic limbs of urethenised cats

Cat No.	Minutes after injection	Amount injected	Gamma of Inulin/ml. of blood			Arterio-venous difference	
			Artery	Isothermic vein	Hypothermic vein	Isothermic limb	Hypothermic limb
1	5 min.	1 gm./kg.	251–36°C	225–36°C	183–27°C	—26	—68
	50 min.	-do-	121–36°C	103–36°C	133–27°C	—19	+12
2	5 min.	1 gm./kg.	304–36°C	288–36°C	194–26°C	—16	—100
	50 min.	-do-	116–36°C	105–36°C	121–26°C	—13	+5
3	5 min.	0.5 gm./kg.	182–35°C	141–35°C	103–27°C	—41	—79
4	5 min.	0.5 gm./kg.	170–36°C	152–36°C	110–28°C	—18	—60
	120 min.	-do-	103–36°C	98–36°C	100–28°C	—5	—3

TABLE III

Study of serum or plasma protein in hypothermic animal

Expt. Animal No.		Method of hypothermia	Rectal temp. C°	Total Pr. gm.%	Alb. gm.%	Total Glob. gm.%	Globulin gm. %					Fibri-nogen	% decrease from normal level in			A/G
							Aplha1	Alpha2	Alpha3	Beta	Gamma		Total Pr.	Alb.	Total Glob.	
1	Cat	S.C.	35°C	6.87	2.46	3.99	0.46	1.11	0.45	0.73	1.24	0.42	11.9	8.1	19.5	0.61
			25°C	6.05	2.26	3.21	0.40	0.92	0.38	0.64	0.87	0.58				0.70
2	Cat	S.C.	37°C	7.43	3.16	4.27	0.65	1.02	0.51	0.95	0.71	0.43	9.3	20.9	0.5	0.82
			25°C	6.75	2.50	4.25	0.52	0.92	0.63	0.90	0.70	0.58				0.68
3	Cat	S.C.	36°C	7.13	2.13	5.0		1.76		1.43	1.79		11.6	14.6	0.4	0.426
			30°C	6.3	1.82	4.48		1.61		1.61	1.26					0.410
4	Cat	S.C.	36°C	7.80	2.0	5.8	0.60	1.91	0.92	1.38	0.99		3.7	.9	1.9	0.344
			30°C	7.52	1.82	5.69	0.49	1.89	0.83	1.65	0.83		8.7	20.5	4.7	0.319
			25°C	7.12	1.59	5.53	0.52	1.86	0.76	1.53	0.66					0.287
5	Cat	S.C.	36°C	7.23	2.53	4.70	0.43	1.04	0.62	0.83	1.78		7.3	20.1	1.5	0.538
			28°C	6.70	2.02	4.63	0.46	0.98	0.59	0.76	1.89					0.431
6	Cat	E.C.C.	36°C	8.81	2.59	5.67	0.31		1.38	2.70	1.29	0.55	7.6	19.7	1.1	0.457
			32°C	8.14	2.08	5.63	0.35		1.35	2.65	1.29	0.43	17.4	24.3	9.2	0.369
			28°C	7.50	1.96	5.15	0.32		1.22	2.58	1.03	0.39				0.380
7	Cat	E.C.C.	36.5°C	7.68	3.04	4.18	0.36	1.05	0.55	0.99	1.23	0.46	12.4	18.4	8.1	0.727
			28.8°C	6.73	2.51	3.83	0.30	0.96	0.59	0.95	1.04	0.38				0.651
8	Cat	S.C.	36°C	7.70	2.14	5.56	0.38		2.23	1.28	1.67		4.1	nil	5.4	0.387
			30°C	7.38	2.13	5.25	0.47		1.62	1.19	1.96		12.2	nil	15.5	0.405
			28°C	6.76	2.13	4.68	0.57		1.41	1.13	1.52					0.460
9	Dog	S.C.	34°C	6.50	2.33	4.17	0.27	0.87		2.76	0.27		12.1	14.6	11	0.559
			27°C	5.71	1.99	3.72	0.27	0.81		2.30	0.43					0.535
10	Dog	S.C.	32°C	5.37	1.98	3.39	0.25	1.01		1.57	0.56		17.7	27.8	11.8	0.585
			26°C	4.42	1.43	2.99	0.21	0.80		1.68	0.30					0.478

S.C.—Surface Cooling ;   E.C.C.—Extracorporal Cooling ;   A/G—Albumin/Globulin.

REFERENCES

Brody, S. (1945).   Bioenergetics and Growth, pp. 281-287, Reinhold Publishing Co.   New York.

Drury, H. F. (1948).   Identification and estimation of pentoses in the presence of glucose. *Arch. Biochem.*, **19**, 455.

Hawk, P. B., Oser, B. L., and Summerson, W. H. (1954).   Practical Physiological Chemistry. 13th ed. p. 602.

Little, J. M. (1949).   A modified diphenylamine procedure for the determination of Inulin. *J. Biol. Chem.*, **180**, 747

Mukherjee, S. R., Maitra, S. R., Dey, P. K., Roy, A., Maiti, A. K., Roychowdhury, M. K., Chowdhury, A. with collaboration of Sen, P. B., Das, N. N., and Sarkar, B. B. (1956). Hypothermia induced by cooling blood in extracorporeal circuit. *Ind. J. Physiol. & Allied Sci.*, **10**, 185.

Mukherjee, S. R., Roy, B., Sen. H. K. in collaboration with Roy, A., Dey, P. K., and Banerjee, S. (1957).   Studies in plasma and blood volume and haematocrit before and during hypothermia using extracorporeal circuit and after rewarming (using I131 human serum albumin). *J. Expt. Med. Sci.*, **1**, 47.

Robbin, J., and Roll, J. E. (1957).   Recent progress in hormone research.   Pincus, G. (Ed.), **13**, 161-208.

Sandberg, A. A. *et al.* (1957).   *ibid.*, Pincus, G (Ed.), **13**, 209-267.   Academic Press Inc., New York.

Sen, P. B., Dey, P. K., Maity, A. K., Roy, A., Chowdhury, A., Roychowdhury, N. K., Das, N. N., Sarkar, B. B., Maitra, S. R., and Mukherjee, S. R. (1957).   Carbohydrate tolerance in hypothermia, *Ind. J. Physiol. & Allied Sci.*, **9**, 13.

VASCULAR ANATOMY OF THE FLOWER OF *SPHENOCLEA ZEYLANICA* GAERTN. AND SOME OTHER RELATED SPECIES*

by D. P. GUPTA, *School of Plant Morphology, Meerut*

(Communicated by V. Puri, F.N.I.)

ABSTRACT

External morphology and vascular anatomy of the flower of *Sphenoclea zeylanica* along with three species of *Lobelia* and two species of *Campanula* have been studied.

The sepals in *S. zeylanica* and *C. latifolia* are one trace organs, the marginal bundles having been derived as branches from the median bundles. In *Lobelia* species and *Campanula canescens*, on the other hand, sepal marginal bundles arise conjoint with petal midrib bundles.

In *Sphenoclea* the placental bundles are amphicribral while in *Campanula* there is a ring of vascular tissue with endarch orientation which gives off branches to the placenta. In *Lobelia*, on the other hand, there is a solid protostele which supplies the placenta.

The placental bundles in *Sphenoclea* are completely utilized in supplying the placenta while in *Campanula* and *Lobelia* species some remnants of placental strands continue up to the stigma.

The present anatomical studies do not render any support for the separation of *Sphenoclea* from *Lobelia* and *Campanula*.

INTRODUCTION

The systematic position of the genus *Sphenoclea* has long attracted the attention of botanists. While Engler (1897) and Hutchinson (1926) placed it under a special section campanuloideae, Airy Shaw(1948) raised it to rank of a separate family and suggested relationship with the Phytolaccaceae. Subramanyam (1950), on the basis of his embryological studies, supported a separate family for this genus, but he did not envisage any relationship with either the Phytolaccaceae or the Primulaceae, as was suggested by Airy Shaw(1948).

As nothing is known about the vascular anatomy of the flower of *Sphenoclea* and its so called relatives, on Professor V. Puri's suggestion, the floral anatomy of this genus, and, for comparison, species of some other genera available have been investigated.

MATERIAL AND METHODS

In all six species, e.g. *Sphenoclea zeylanica* Gaertn., *Lobelia syphilitica* Lin n., *L. cardinalis* Linn., *L. erinus* Linn., *Campanula latifolia* Linn. and *Campanula canescens* Wall. belonging to three genera have been studied. The material of *Sphenoclea* was collected from a pond about four miles north of Meerut and that of *Lobelia erinus* from a local garden. *Campanula latifolia* and *Campanula canescens* were secured from the departmental collections. The material of two species of *Lobelia* (*L. syphilitica* and *L. cardinalis*) were given to me by Prof. V. Puri from his collection, made in U. S. A. in 1950.

All these materials were fixed in F. A. A. and passed through alcohol, xylol series after washing thoroughly in running water. Each material was embedded

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in paraffin, and cut into serial sections, 10–12 microns thick. The slides were stained with crystal violet and erythrosin.

OBSERVATIONS

Sphenoclea zeylanica Gaertn.

The Flower and its Vascular Anatomy:—The flowers are sessile, bracteate and bracteolate arranged densely in lateral and terminal spikes. The sepals are fused at the base and the corolla is campanulate with five lobes. The five stamens are epipetalous and alternate with petals. The ovary is inferior and bilocular. In each locule there are many ovules on stalked placenta that is obviously axile. Style is short with bilobed stigma.

At the base of the flower there is a small plexus of vascular tissue (Fig.2) from which three traces diverge out, one for the bract and two for bractecles (Figs.3–4, br.brl). A little higher up, some ten or twelve traces diverge out from this plexus leaving a small amout of vascular tissue in the centre (Fig.5). In this tissue the xylem elements are scattered and surrounded by phloem. At a higher level, this central mass organizes itself in 3–5 (four being more common) amphicribral vascular bundles with xylem in the centre. These are usually arranged in pairs on opposite sides (Figs.6–7). But where there are only three, the odd bundle is larger than the other two, and occurs on one side, the other two being on the opposite side. It is interesting to note that while they are amphicribral as a rule, they become inversely oriented in some cases. The position and behaviour of these bundles clearly indicate that they are ventral bundles of the carpels.

In a few cases, the central plexus organises itself into two concentric vascular bundles with xylem in the centre and phloem all round. These are situated on the septal radii and not laterally on either side. A little higher up, each of these bundles splits into two branches which traverse outward towards the placenta of their respective side. These are apparently the ventral bundles of the carpels that remain fused in pairs in the lower region.

In the ovule-bearing region the carpellary ventrals diverge out into the corresponding placentae which are well developed, pendent and are as long as broad (Figs.7–9, Cv). They are completely utilized in giving out many ovular traces, Further up, the placentae split apart in such a way that two half placentae of the adjacent carpels remain fused together. An extremely narrow channel links up the two locules, and the ovary becomes unilocular (Figs. 10–11). Beyond the placental region the septae again fuse resulting in bilocular condition (Fig.12).

The ten peripheral bundles in the ovary wall distribute themselves equally in two carpels. In cases where there are twelve peripheral bundles, seven pass into one carpel and five into the other.

The ten peripheral bundles constitute the vascular supply of the remaining floral organs. Five of these, situated on sepal radii, split up tangentially into two each (Figs.10–11). While the outer ones of these daughter branches (Fig.10, Sp) form the sepal midrib bundles, the inner ones, after giving a branch each to the ovary "wall" (Fig. 12, sl), are destined to supply the antisepalous stamens (Fig. 11, St). The sepal midrib bundles also give off some branches laterally (Fig. 14, Sm). The other five peripheral bundles that are situated on petal radii give off some branches to the ovary "wall" (Fig. 12, sl) and the rest form the petal midrib bundles, each of which sends off one branch on either side into the marginal region of the petal (Figs. 15, 16, pt. pm). The branches in the ovary wall traverse into the style and end in the stigmatic region (Figs.15–18).

The condition described above holds good for cases where there are only ten peripheral bundles in the "ovary wall". It will be recalled that in some cases there are twelve such bundles which distribute themselves somewhat differently. Two
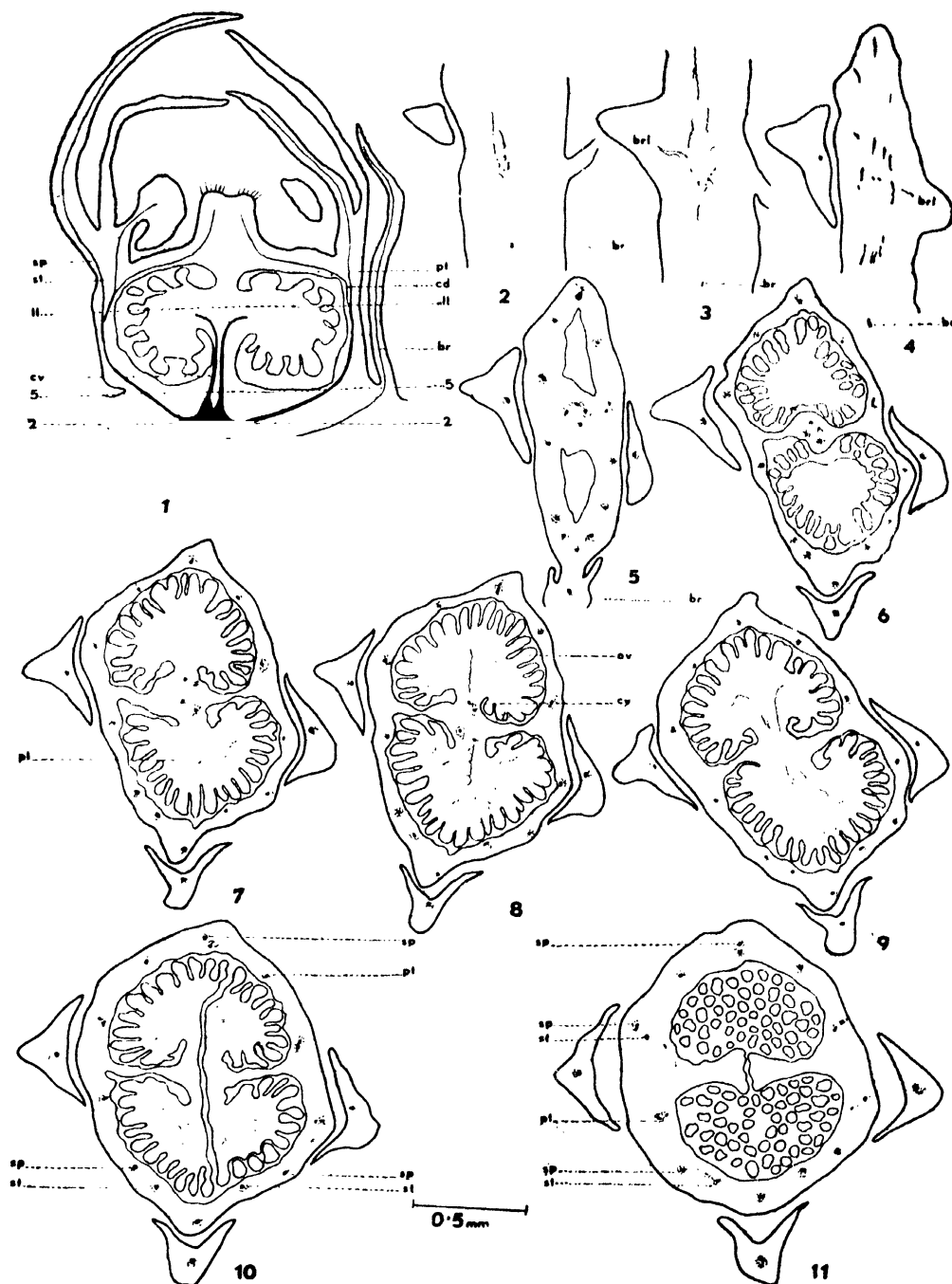
TEXT-FIG. 1.

Figs. 1–11. *Sphenoclea zeylanica.* Fig. 1. Semi-diagrammatic longitudinal section of flower showing vascular supply to different floral organs. Numbers indicate levels at which transverse sections have been shown in figures bearing the same number. Figs. 2–11. Serial transverse sections of the flower from base upward. Note the migration of carpellary ventrals into the placenta in Fig. 8.

Br–bract trace ; *Brl*–the trace for bracteoles ; *ov*–ovule ; *cv*–carpellary ventrals ; *sp*–sepal trace ; *pt*–petal trace ; *St*–staminal trace ; *cd*–carpellary dorsal ; *pl*–placental lobe.

of the bundles pass out directly into two stamens and two other adjoining bundles enter directly into two sepals without giving off any staminal trace (Fig. 13, Sp.St.). Thus, this increase in the number of peripheral bundles seems to have been brought about by a very early separation of the two staminal bundles from their corresponding sepal midrib bundles with which they are usually fused up to a higher level.
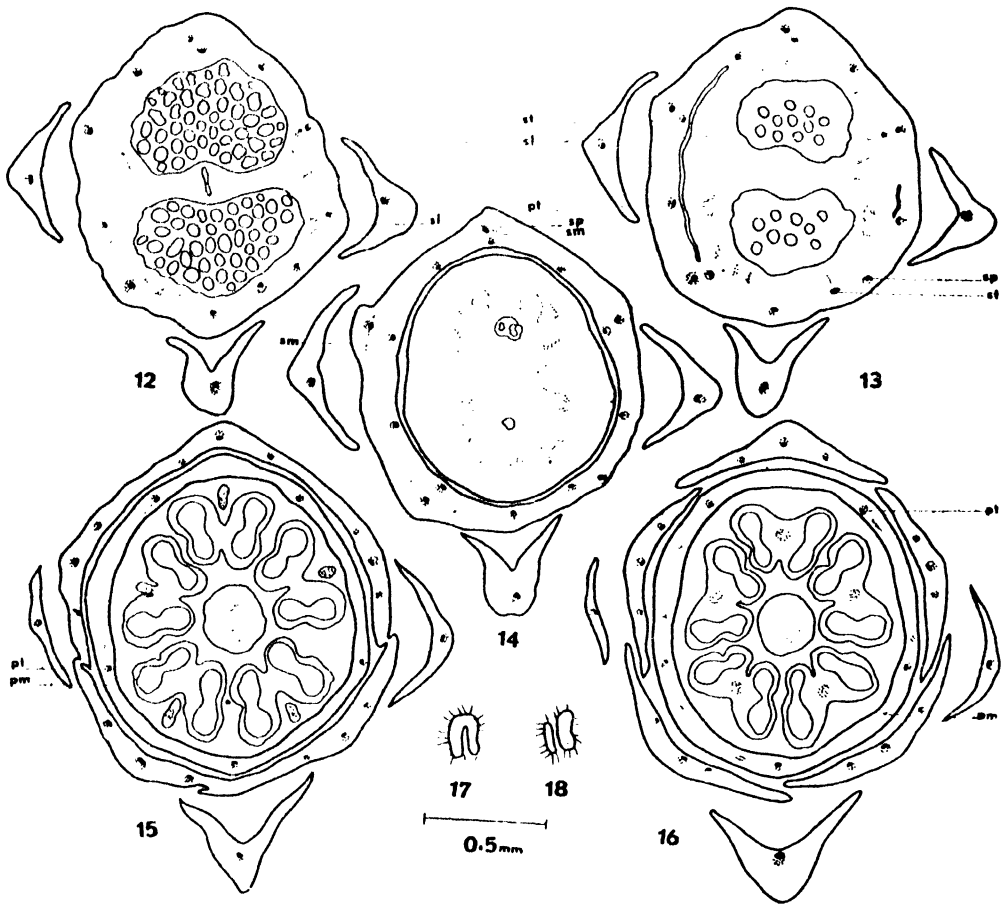
TEXT-FIG. 2

Figs. 12–16. *S. zeylanica*. Serial transverse sections of the flower (cont.). Figs. 17–18. Transverse sections of the stigma.
Sl–secondary laterals ; *Sm*–trace for sepal margins ; *Pm*–trace for petal margin.

Lobelia

The Flower and its Vascular Anatomy :—The three species studied are *L. syphilitica* Linn., *L. cardinalis* Linn., and *L. erinus* Linn. The pedicellate flowers of *Lobelia* are arranged in simple racemes. They are bracteate, each with an inferior bilocular ovary having numerous ovules on axile placenta. The calyx is five-partite and the corolla is obliquely bilipped, the upper lip being two-partite and lower three-lobed. There are five stamens with syngenesious anthers forming a ring round the style.
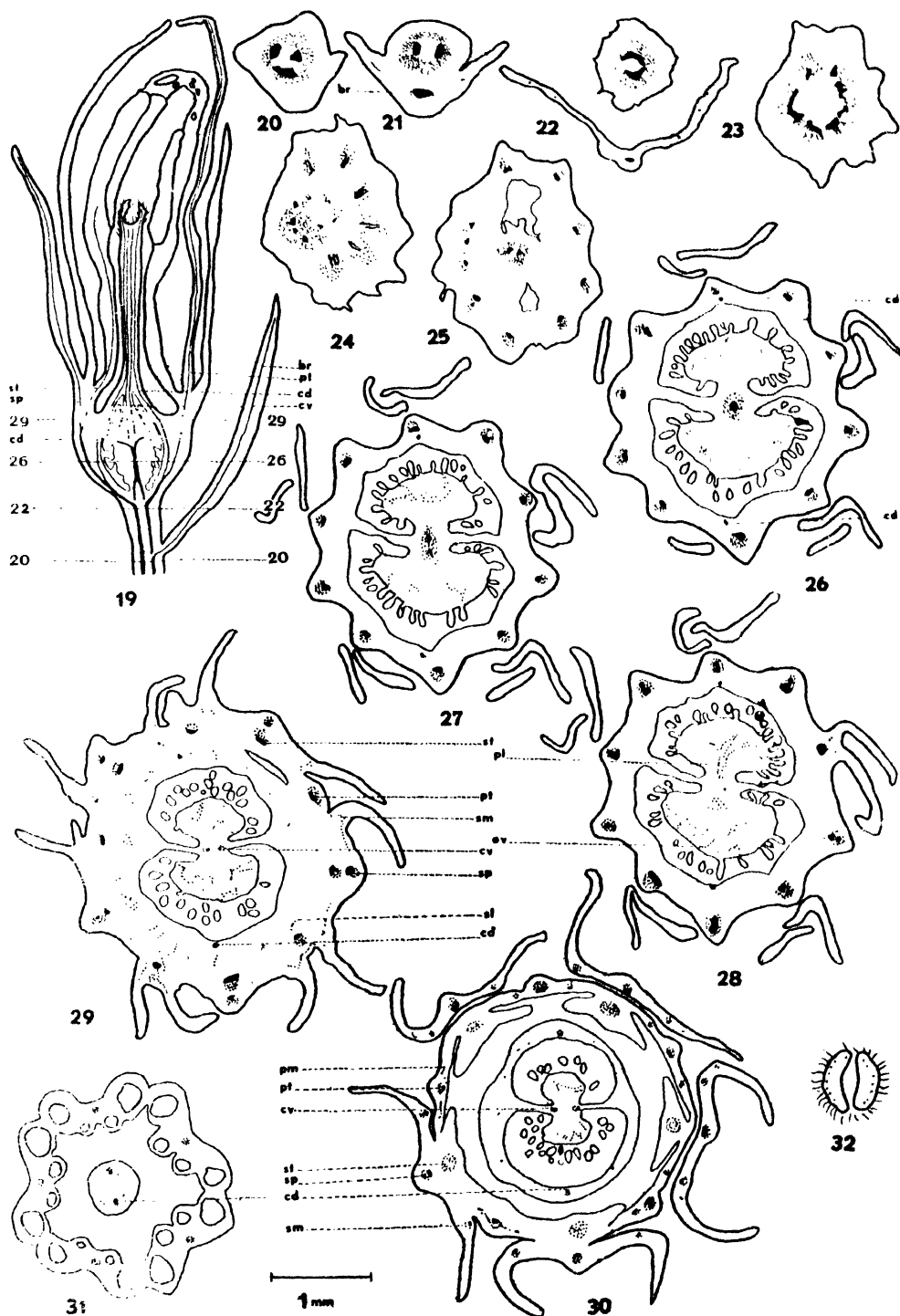
TEXT-FIG. 3

Figs. 19-32. *Lobelia syphilitica.* Fig. 19. Semi-diagrammatic longitudinal section o the flower showing vascular supply to different floral organs. The numbers indicate the levels at which transverse sections have been shown in figures bearing the same numbers. Figs. 20-31. Serial transverse sections of the flower from pedicel upward. Fig. 32. T.S. of the stigma.

The pedicel of *Lobelia syphilitica* contains a more or less complete ring of vascular tissue with xylem differentiated at three places (Fig.20).   A little higher up a trace involving one of the xylem groups diverges out through the cortex and enters the subtending bract in which it divides further into some branches (Fig.21, br).   After passing out the bract trace a ring of vascular tissue is formed (Figs. 22, 23).

Higher up, somet en unequal traces pass out from the ring (Figs.23, 24).   They traverse obliquely upward from the receptacle and supply the peripheral organs at a higher level.   A small amount of vascular tissue in the form of a ring is left in the centre (Fig.25). As the ovary cavity is about to appear, the central vascular tissue becomes compact and protostelic with phloem surrounding the xylem (Fig.26). From this solid vascular core a branch is given out to a placenta on either side (Fig.27).   Herein, it breaks up into small ramifications to form the vascular supply of the ovules (Fig.28, ov).   In some flowers the solid vascular core splits up in the very beginning into two parts, each of which gives off a branch to its corresponding placenta.   This branch ramifies and forms the vascular supply of the ovules.   Beyond the ovule-bearing region the septa splits up in the middle and the ovary becomes unilocular.

After supplying the placentae some vascular tissue is left at two places in the centre.   These strands migrate into the corresponding septal regions, and in doing so, become inversely oriented (Figs.29, 30, Cv) and continue upward into the style for varying height along with two other bundles.   The latter two, occurring one on each side and in a plane at right angles to the septum in the lower region of the flower, are obviously the carpellary dorsals which have separated from the peripheral bundles during their upward course (Fig.26, cd).   These bundles after traversing the style ramify profusely in the bilobed stigma, and thus disappear (Fig.32).   The carpellary ventral traverse the style, upto varying heights, and branches in the upper region of the style.   Each stigmatic lobe bears many hair-like processes.

The ten peripheral bundles form the vascular supply of the peripheral organs: two of these give the carpellary dorsals in the lower region.   Five of these, occurring on sepal radii, split up tangentially into two each (Fig.29).   The outer ones of these branches form the sepal midrib bundles while the inner ones, after giving off some branches to the carpellary "wall", supply the corresponding antisepalous stamens. The other five alternating bundles, occurring on petal radii, give off two branches each, one to each of the adjacent sepal margins (Fig.29, pt, sm).   Besides, they also send some branches to the carpellary "wall" (Fig.29, sl).   The remaining portions form the petal midrib bundles.   Each petal midrib bundle gives two branches to each of its margins (Fig.30, Pm).   The sepal midrib bundle branches further in the upper region.   The branches in the carpellary "wall" are the secondary laterals which may continue into the style for varying heights.

The condition in *L. cardinalis* flower is exactly similar to that in *L. syphilitica*. The pedicel of *L. erinus* also has a complete ring which gives off only six traces. These latter split up into ten peripheral bundles, as they traverse obliquely upward. In other respects the condition in this species is the same as in *L. syphilitica* and *L. cardinalis*.

<center>*Campanula*</center>

The Flower and its Vascular Anatomy :—The flowers in the two species of campanula, *C.canescens* Wall., and *C. latifolia* Linn. are sub-sessile. They are arranged in panicled clusters (*C. canescens*), or simple racemes (*C. latifolia*).   The calyx is deeply five lobed, and the corolla is campanulate, usually five fid.   The stamens are five, free with filaments dialated at the base.   The ovary is inferior with three locules, each having many ovules on axile placentae.   The style is cylindrical, and the stigma is shortly tri-lobed.
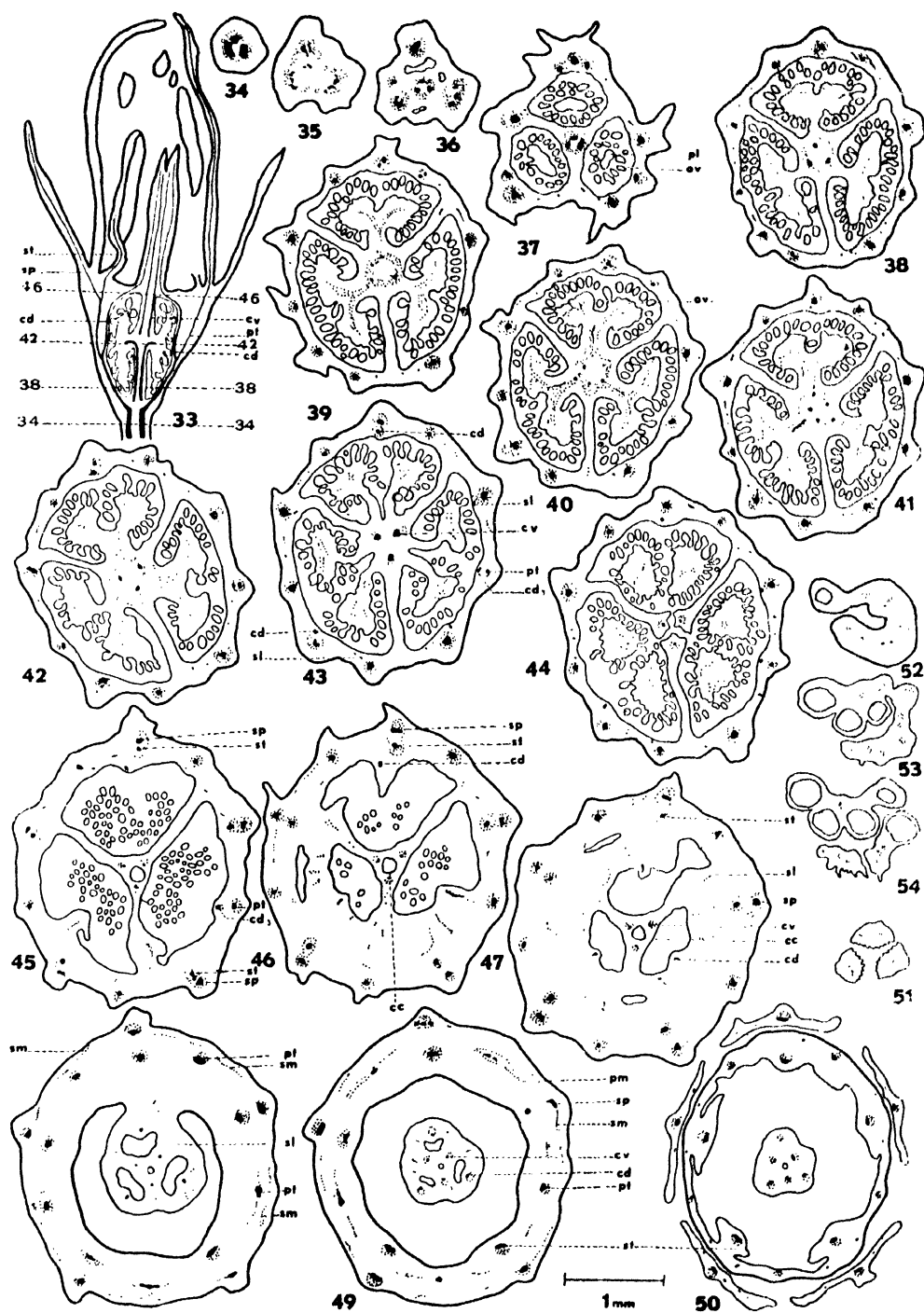
Text-fig. 4

Figs. 33–54. *Campanula canescens.* Fig. 33. Semi-diagrammatic longitudinal section of the flower showing vascular supply to different floral organs. Figs. 34–50. Serial transverse sections of the flower from pedicel upward. Fig. 40. Shows the bifurcation of placental strands. Note the splitting of the placenta in Fig. 43. Fig. 51. T. S. of the stigma. Figs. 52–54. Serial transverse sections of the style showing staminal outgrowth bearing anther loculi.

The stele in the pedicel of the flower of *Campanula canescens* is more or less a complete ring (Fig.34). From this ring three unequal traces pass out to the periphery leaving a small amount of vascular tissue in the centre. At this stage the pedicel becomes trilobed and each lobe is supplied with a single trace (Fig.35). In one lobe the vascular strands split up radially into three parts. The middle of these is smaller than the other two (Fig.36). In the case of second lobe the vascular strands divide only into two branches which behave differently (Fig.36). One of them splits up into three, the middle of these being larger than the other two. The other branch remains undivided. This lobe, therefore, has four vascular bundles (Fig.38). In the third lobe also the vascular strands divide radially into three parts but in this the middle one is larger than the other two (Fig.37). Thus, in all, a ring of ten vascular bundles is formed (Fig.38).

These ten peripheral vascular bundles constitute the vascular supply of the different floral organs. Five of these are situated on the sepal radii. These bundles give off branches to the inner side, two of which form the carpellary dorsals, while rest form the secondary laterals (Fig.43, cd, sl). The third carpellary dorsal is received from the bundle situated on the petal radii (Fig.43, cd₃). After supplying the carpellary wall, each of the five bundles splits up tangentially into two, the inner one of which forms the staminal bundle and the outer one the sepal midrib bundle (Figs.45, 46, Sp. St).

The other five bundles situated on petal radii, send off two branches, each to the margins of adjacent sepals and the rest form the petal midrib bundles (Figs.48, 49, Sm. pt). Each petal midrib bundle soon gives out two lateral branches for the margins (Fig.49, Pm). The five bundles situated on petal radii also send a number of branches to the inner side. One of these forms the third carpellary dorsal, the two others having been received from bundles on sepal radii (Fig.43, cd₃), while others form secondary laterals (Fig.43, cd, sl).

The vascular tissue which is left in the centre, forms a complete ring of vascular cylinder with endarch xylem. The xylem is interrupted and is better developed at three places where the placentae are to develop later on while phloem forms a complete ring (Fig.38). The placentae hang downward and so their lobes appear in the ovary cavities before their attachment (Fig.37, pl). The central cylinder gives out three branches, one to each placenta. This branch splits up into two, and these traverse into the placental lobes of their respective sides, the placenta being bilobed (Figs.39–42). Each branch gives out many ovular traces (Fig.40, ov). The passing out of these three traces leaves only three bundles in the parent stele, and these occur on septal radii. They have normal orientation (Fig.43, cv). In the upper region the whole placental mass splits up in such a way that half placentae of two adjacent carpels remain fused. Thus the ovary becomes unilocular (Fig.44). Beyond the ovule bearing region the septae again fuse together resulting in a trilocular ovary once more (Fig.45). A trilobed cavity, the stylar canal, is also enclosed in the centre. This cavity becomes circular and continues up to varying heights within the style (Fig.47, cc).

The three central bundles left after the departure of the placental strands traverse along with the carpellary dorsals into the style (Figs.49, 50, cd. cv). In its upper region these bundles ramify profusely and supply the three lobes of the stigma (Fig.51). Each stigmatic lobe has hairs on its inner side. Some trichomes are also present on the ovary wall and the petals.

In a single case the gynaeceum, after bearing the ovules in the lower region, proliferates and bears anthers in the upper region (Figs.52–54).

Campanula latifolia differs from *C. canescens* in that the vascular cylinder of the pedicel of *C. latifolia* gives off four traces to the periphery. Three of these divide radially into three each and occupy the three angles of the pedicel which has in the meantime become trilobed in transverse section, the fourth remaining undivided on one side. In *C. canescens* only three traces are given off from the vascular cylinder

of the pedicel. Out of these, two divide into three each and one divides first into two parts. One of these latter again divides into three. Thus finally a ring of ten vascular bundles is formed in the periphery of both the genera.

The sepal supply of *C. latifolia* differs somewhat from that of *C. canescens*. In *Campanula latifolia* the sepal midrib supplies branches to its margins. In *C. canescens* the petal midrib supplies the two margins of adjacent sepals. In other respects it is similar to *C. canescens*.

CONCLUSIONS

As pointed out earlier this study was undertaken with the definite purpose of comparing the vascular anatomy of the flower of *Sphenoclea* with that of other species. There are some points in which *Sphenoclea zeylanica* differs from others.

As is well known the flowers in *Sphenoclea* are sessile and those of *Campanula* and *Lobelia* are stalked. In addition, the flowers of *Sphenoclea* have bracts and bracteoles, while *Lobelia* has only bracts, and *Campanula* lacks both bracts and bracteoles.

The sepals in *Sphenoclea zeylanica* are essentially 1-trace organs, the marginal bundles having been derived as branches from the median bundles. In *Lobelia* species and *Campanula canescens*, on the other hand, the sepal marginal bundles arise conjoint with petal midrib bundles. *Campanula latifolia*, however, resembles *Sphenoclea* in this respect.

Sphenoclea zeylanica differs from other genera in the vascular supply of the placenta. In this species the marginal bundles which supply the placenta are amphicribral. In some cases they may be inversely oriented. These bundles diverge out into the corresponding placenta, and are completely utilized in the formation of ovular supply. In *Lobelia* the placental strands form a compact solid core of vascular tissue. This solid core supplies branches to both the placentae, and the remainder continues up to the stigma. Only in the genus *Campanula* the placental strands are normally oriented and form a ring, and this supplies the placentae. Whatever remains of this ring continues up to the stigma.

The occurrence of normally oriented placental strands in *Campanula* deserves some attention. Usually in axile placentation, the placental strands are inversely oriented with respect to the floral axis (see Puri, 1951, 1952). What can be the possible significance of this feature is difficult to say. But a similar situation has also been reported in *Dianthus, Silene, Sedum* and *Azalea* (Henslow, 1891). Subramanyam (1955) recently re-investigated the condition in *Sedum*, and he has shown that the placental strands are actually inversely oriented in this genus. Other genera also need re-investigation in this respect. But the material of *Campanula* that has been studied here, definitely shows normally oriented placental strands.

Thus we see that the differences in the vasculature of the flower of *Sphenoclea, Campanula* and *Lobelia* are not of much importance. They are apparently just minor differences and as such they do not provide any support for the separation of *Sphenoclea* from the Campanulaceae.

REFERENCES

Airy Shaw, H. K. (1948). 'Sphenocleaceae' in Flora Malesiana. Ser. I, **4**, 27–28.
Engler, A. (1897). Die naturlichen Pflanzenfamilien **4**, Abt. 4 und 5, Berlin.

Henslow, G. (1891).   On the vascular systems of floral organs and their importance in the
    interpretation of the morphology of flowers.   *J. Linn. Soc. Bot.* London, **28**, 151–97.
Hutchinson, J. (1926).   The Families of Flowering Plants.   London.
Puri, V. (1951).   The role of floral anatomy in the solution of morphological problems.
    Bot. Rev., **17**,, 471–553.
———— (1952).   Placentation in Angiosperms.   *Bot. Rev.*, **18**, 603–651.
Subramanyam, K. (1950).   A contribution to our knowledge of systematic position of Spheno-
    cleaceae.   *Proc. Ind. Acad. Sci.*, Sec. B, **31**, 60-65.
———— (1955).   Morphological studies of some species of Sedum.   *Amer. J. Bot.*, **42**, 850-55.

THE OVIPOSITION BEHAVIOUR OF *BAGRADA CRUCIFERARUM* KIRKALDY (PENTATOMIDAE : HETEROPTERA) AND THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE SPEED OF DEVELOPMENT OF EGGS

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(Communicated by Hem Singh Pruthi, F.N.I.)

ABSTRACT

In the laboratory, the *Begrada cruciferarum* females prefer to lay eggs in a soft medium such as cotton wool.

The duration of egg stage at 30, 35 and 40°C, is 95 5, 72.1 and 74.4 hours respectively. There is no hatching at 45°C. Relative humidity, ranging from 20 to 80 per cent, has no influence on the speed of development and the viability of eggs.

The pentatomid, *Bagrada cruciferarum* Kirkaldy, feeds on cruciferous plants particularly radish seed pods. The pest is most active in the field during April and May and by the end of May large number of adults die due to the high atmospheric temperature. The characteristic feature of this pest is the rapid multiplication within these two months. There are comparatively very few bugs seen before and after that period. With a view to investigate the factors that contribute to the rapid increase in their number, the preference of ovipositing females to various surfaces and the influence of temperature and humidity on the speed of development of eggs was studied and the results are presented in this paper.

Preference of various surfaces for oviposition

In the laboratory ovipositing females were offered a number of surfaces of various materials, to lay eggs on them, viz., medium quality green and white cloth, green and white filter paper, 0.2 inch thick layers of dry and wet cotton wool, soil clods of 0.2 to 0.3 inch diameter, and coarsely powdered dry leaves and seed pods of radish. Circular discs of 1.9 inches in diameter were cut out of these materials for the purpose. The soil clods and powdered dry leaves and seed pods were placed in dishes of the same diameter. The 8 types of surfaces tested were fixed on the floor of a cage 12″×9″×9″ with pins. The surfaces were placed at random, at equal distance from one another. Two hundred insects of either sex were released in the cage which was placed in subdued light in the corner of a room for 4 hours from 1.0 p.m. to 5.0 p.m. At 5.0 p.m. the discs and dishes were taken out of the cages and were then left aside for 48 hours. When fresh, the eggs are dirty white or slightly greyish white, but after 48 hours their colour becomes orange red, and as such eggs are easy to count. The experiment was conducted at 30°C in 60 per cent relative humidity.

The analysis of variance showed that the effect due to the various surfaces was significant at the 1 per cent level (the least significant difference was 14.1; Table I). Compared to the number of eggs laid on dry leaves and seed pods as control, that laid on dry cotton wool was significantly higher, whereas the differences between other surfaces were not significant. It was noticed that if the cotton

wool was too wet, a female did not lay eggs. The fact that as many eggs were laid on soil or in dry leaves and seed pods and that far more eggs were laid on white cotton wool is a pointer that colour of the surface is only of secondary importance, the primary factor being the texture. In the field, egg laying females prefer the light coloured but loose and soft debris to the green radish plants.

TABLE I

Number of eggs laid by 200 Bagrada cruciferarum *in 4 hours*

Type of surface	Eggs (Average of 4 observations)
White filter paper	1.00
Green filter paper	1.5
White muslin 35 meshes	2.7
Green muslin 35 meshes	11.0
Cotton wool dry	42.7**
Cotton wool wet	17.7
Soil clods in a dish 3″ in diameter	8.2
Dry leaves and seed pods in a dish 3″ in diameter (control)	11.7

L.S.D. = 14.1

**Significant at the 1 per cent level; least significant difference is 14.1.

Influence of temperature and humidity on the duration of egg stage

Eggs laid within 2 hours ($\pm$ 1 hr.) were incubated at 30, 35, 40 and 45°C in 20, 40, 60 and 80 per cent relative humidities. The humidity was controlled by appropriate strengths of sulphuric acid poured at the bottom of one pound glass jars. Fifty eggs were placed on a filter paper in a petri dish and the dish was placed on a glass tripod made to stand in the jar. The jar was sealed with a plastic screw cap and placed in an incubator at the required temperature. The number of nymphs hatched were recorded after every two hours and the end of egg stage was the mid-time between the two observations. There was no hatching at 45°C, hence that temperature is omitted from the results presented in Table II.

The data were analysed as per single randomised block. The effect of temperature was highly significant at the 1 per cent level, whereas that due to humidity was just significant at the 5 per cent level (Table II). Since the difference between the egg stage at various humidities were very small, the largest being only 1.2 hours, these are considered to be due to chance. Except at 45°C, there were no significant differences in mortalities at the different temperatures and humidites tested.

The duration of egg stage at 30°C was 95.5 hours which was significantly higher than 72.1 hours at 35°C and 74.4 hours at 40°C. However, the difference between the last two temperatures was not significant, which indicated that the speed of development was retarded as the temperature of incubation was raised from 35 to 40°C. At 45°C there was no hatching.

TABLE II

Duration of egg stage (hours) at various levels of temperature and humidity

Temperature °C	Percentage of relative humidity				
	20	40	60	80	Mean
30	95.8	96.5	95.2	94.4	95.5**
35	72.5	72.3	72.0	71.7	72.1
40	74.8	74.8	74.2	73.8	74.4
Mean	81.0	81.2	80.5	80.0	80.7

**Effect of temperature significant at the 1 per cent level ; least significant difference 8.3 ; that of humidity not significant.

CONCLUSIONS

1. *Bagrada cruciferarum* Kirkaldy females when given a choice of a number of surfaces in the laboratory prefer to lay eggs in a soft medium such as cotton wool. In the field, they lay most of their eggs in debris composed of dry leaves and seed pods, etc.

2. The speed of development at 35°C was significant than that at 30°C. The speed of development at 40°C was slightly lower than that at 35°C, but the difference was not significant. There is no hatching at 45°C, obviously this temperature is too high for the normal development of eggs.

3. Relative humidities of 20 to 80 per cent have no influence on the speed of development of eggs. Within this range, moisture does not influence the viability of eggs.

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REFERENCES.

Fisher, R.A., and Yates, F. (1948). Statistical Tables for Biological, Agricultural and Medical Research.   (Oliver and Boyd : London.)

THE SKIPPER–FROG AS A SUITABLE EMBRYOLOGICAL ANIMAL AND AN ACCOUNT OF THE ACTION OF MAMMALIAN HORMONES ON SPAWNING THE SAME

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Abstract

There are three common species of ranids in South India, viz., *Rana hexadactyla* Lesson (the green-frog), *R. tigrina* Daud. (the bull-frog) and *R. cyanophlyctis* Schn. (the skipper-frog). Of these, the bull-frog hibernates during winter and aestivates during summer. The green-frog exhibits a short winter spawning in addition to the major one during the monsoon showers. For artificially spawning and getting ripe eggs for embryological studies, the larger species are difficult to handle and they are also becoming scarce around Bangalore. The skipper-frog is easy to handle and during the monsoon season, a parenteral injection of 2-3 gravid pituitary glands from the same species will make the gravid females yield ripe eggs ; during winter, they require 4-5 glands. The time taken for the injected pituitary gonadotrophins to act is about 12-14 hr. during winter and 9½-10 hr. during the breeding season. Five gravid catfish (*Heteropneustes*) pituitary glands also ripen the eggs of the skipper-frog and frog pituitaries are known to act reciprocally. Mammalian hormones like follicle-stimulating and luteinizing hormones, pregnant mare serum, chorionic gonadotrophin, ostrogen, cortisone, adrenocorticotrophic hormone, growth hormone, thyroid-stimulating hormone and oxytocin have no effect on the frog's ovary ; androgen, progesterone and desoxycorticosterone acetate bring about ovulation (oviposition) in the skipper-frog. All the above mentioned hormones, except oxytocin, bring about ovulation in frog when combined with a threshold pituitary gland dose, viz., two gravid pituitary glands during the non-breeding season. A table showing the normal stages in the development of *Rana cyanophlyctis* is also appended. The skipper-frog, therefore, is recommended as a suitable animal for embryological studies.

Introduction

In most Indian universities, demonstration of frog embryology has not been undertaken as the technique of spawning and fertilizing the eggs under laboratory conditions to follow up their further fate is not adequately developed. With a view to provide the correct dosage of pituitary glands for implantation or injection so as to induce spawning in frog and also to study the effect of mammalian hormones towards this end, we have conducted a series of experiments, the results of which are presented here. In this connexion, Rugh (1948) has given all details with regard to inducing spawning in the American frog *Rana pipiens.*

Material and Methods

The following ranid species available locally have been tested :
1. *Rana hexadactyla* Lesson (the green-frog),
2. *Rana tigrina* Daud. (the bull-frog),
3. *Rana cyanophlyctis* Schn. (the skipper-frog).
Pituitary glands taken out of gravid female frogs of the same species (homoplastic) are mashed in a small quantity (1 ml.) of distilled water or standard Holtfreter solution in a homogeniser and injected parenterally. Implantation of pituitary glands into the dorsal lymph sac or into the peritoneal cavity with a

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*Council of Scientific and Industrial Research Scholar.

syringe has not been uniformly followed. In injecting mammalian hormones dissolved in an oil base, we have always administered first the pituitary gland, followed up by the mammalian one after a short interval.

OBSERVATIONS

The three species of frogs, namely, the bull-frog, the green-frog and the skipper-frog which are used for biology demonstrations in most Indian universities could also be utilised for artificially procuring eggs under laboratory conditions for embryological studies. However, the more ubiquitous bull-frogs become scarce during winter when they hibernate and they also aestivate during summer. The females generally emerge to spawn after the first monsoon showers (June). The other two species are available throughout the year. However, the larger of the two species, viz., *hexadactyla* is becoming scarce around Bangalore and is more difficult to handle for stripping purposes. The skipper-frog, like the bull-frog, is cosmopolitan in distribution and being smaller is easy to manipulate and, is therefore, ideally suited for experimental purposes.

The bull-frog has been used successfully for getting eggs during the rainy months, June—September. In the months of April and May (1958), fresh gravid bull-frogs (177-332 gm.) receiving parenteral injections of 4 gravid homoplastic pituitary glands yielded viable eggs normally after 12 hrs.; later in the season (June), a smaller dose (2 or 3 glands) was enough to make them spawn taking about the same time for ovulation. We have always found that if the dose is split, injecting a larger dose first followed by a smaller one after an interval of 10–12 hrs., the frogs yield better results. A very large number of *hexadactyla* glands (seven) are needed to activate the ovary of the bull-frog. During October, uniformly all the bull-frog females showed regressed ovaries. The usefulness of this frog for embryological studies, is therefore, very limited.

The green-frog restricted to South India and Ceylon appears to have two breeding seasons ; a major one during the monsoon months and a very limited winter one (November—January). During May—June, the green-frog requires more than six pituitary glands for making them yield eggs. However, green-frogs receiving 4 glands in combination with 100 I.U. of chorionic gonadotrophin (Antuitrin S : Parke, Davis) yielded a large number of eggs. Test animals receiving four glands in combination with 25 mg. of cortisone (Corlin : Glaxo) yielded fewer eggs.

During the monsoon months, a number of naturally spawning green-frog females were procured and the other gravid ones reacted to pituitary injections very much like the bull-frogs. After the season, quite a number of regressed green-frog females are met with. However, during November—December, these frogs show again ovaries with well developed ova. Quite a few specimens were also procured with ripe ova in the uterus which hatched into tadpoles after fertilization. Obviously, the green-frog has a short winter spawning. We are not aware of any record of winter spawning in the South Indian frogs. During this season, the frogs required 6–8 pituitary glands per injection to yield eggs and the eggs showed up at the cloaca after the lapse of 20–24 hrs.

According to McCann (1932), the thoroughly aquatic skipper-frog may breed at any time of the year in suitable localities. We have never come across a single female during our studies (October 1957—June 1958) showing eggs in the uterus. It was only during the early part of August that a few females with uterine eggs were procured. While all the three species of frogs are available for embryological studies during or just prior to monsoon months, our search for a suitable one during winter months resulted in discovering the skipper-frog ; the green-frog showing a short second spawning season could also be used but the difficulty of securing the specimens and of handling them positively precludes the choice.

During winter months, 4–5 gravid pituitary glands of the skipper-frog mashed in 1 ml. of distilled water and injected intraperitoneally were found effective in making 80–90 per cent of *cyanophlyctis* test animals (39—50 gm.) yield ripe eggs after the lapse of 12–14 hrs. ; in the bigger *hexadactyla* females, it required 24 hrs. after injection for a few eggs to show up at the cloaca when they could conveniently be stripped ; if the day was warm, ovulation took place much earlier than this. We generally injected the female skipper-frog late in the evening, and the next morning after a lapse of 12–14 hrs., the test animals showed up eggs indicating that they were ready for stripping, the laboratory temperature during the winter being 17°—18° C. After fertilization, we have noticed that more than 80 per cent of the eggs hatched out. According to Rugh (1948), the percentage of fecundity increases if the eggs stay in the uterus for 24 hrs. undergoing a physiological maturity. In several of our experiments, we have had more than 95 per cent of the eggs of the skipper-frog hatching out with the eggs having stayed in the uterus for less than 15 hrs., and the animals do not appear to retain the eggs in the uterus for long. During March (day temperature 28° C), we have been able to secure viable eggs after the lapse of 9½–10 hrs., with a four-gland injection and more than 90 per cent of these eggs hatched out into polliwogs when fertilized. We have tested the skipper-frog females for gonadotrophic reactions after the spawning season (September) and the ovary is not completely regressed. Two homoplastic pituitary glands bring about ovulation but the number of eggs is very limited. Probably these are individuals who have already spawned in nature. The reasons that prompted us to select the skipper-frog, therefore, are the short interval that is needed for ripening the eggs, the ease with which the females can be handled and the availability of the gravid females throughout the year even though the skipper-frog eggs are smaller than those of *hexadactyla* and *tigrina*. The eggs of anurans are smaller than those of urodelans and are, therefore, not ideally suited for transplantation studies.
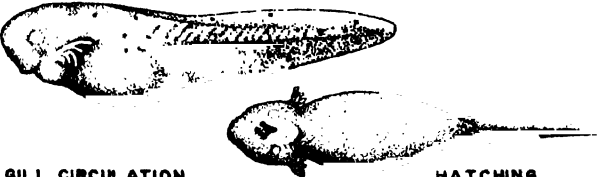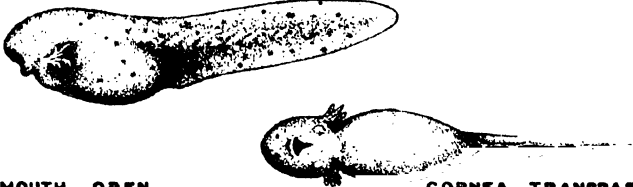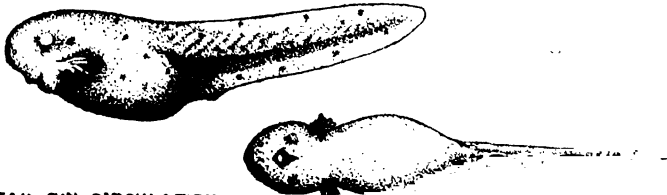
Each skipper-frog female gives at a stripping more than 200 eggs. Further, the skipper-frog also responds to *hexadactyla* pituitary glands ; during November, one or two gravid pituitary glands of the green-frog injected either intraperitoneally or into the dorsal lymph-sac of the skipper-frog made the latter yield viable eggs. Even two pituitary glands of the male *hexadactyla* were effective. However, pituitaries from donors with regressed ovaries did not cause the skipper-frog to ovulate. Moreover, in the absence of spring-water, we have been using stored city water and the eggs undergo normal development in it. For testes mash, we have also been using the same stored water and the sperm are viable, normal saline or standard Holtfreter solution being deleterious.

We have always found that freshly caught specimens of the skipper-frog weighing more than 40 gms., have always given us excellent results ; those stored in the laboratory for more than 8–10 days give indifferent results. Rugh (1948) also recorded that ovarian eggs of frog undergo degeneration rapidly at room temperature.
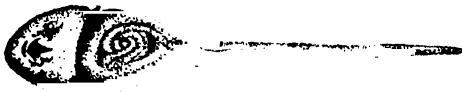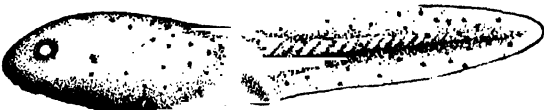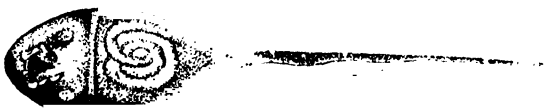
We have also tried, during November, the reciprocal effect of *Heteropneustes* (catfish) and skipper-frog pituitaries on ripening the eggs of each other. Parenteral injection of 5 gravid catfish pituitary glands into gravid skipper-frog females made the latter yield viable eggs ; similarly, 5 gravid pituitary glands of the skipper-frog made the catfish yield viable eggs. In each case, after fertilization, embryos hatched out. It is interesting to note in this connexion that Creaser and Gorbman (1939) recorded of a single instance of fish pituitary successfully spawning frog. Atz (Pickford and Atz, 1957) alluded to the spawning of frog with fish pituitary as a 'feat' accomplished by Wills, Riley and Stubbs (1933), Stroganov and Alpatov (1951) and partially by Atz and Pickford (Pickford and Atz 1957).

STAGE NUMBER			STAGE NUMBER			STAGE NUMBER		
	AGE IN HOURS AT 18°-20°C			AGE IN HOURS AT 18°-20°C			AGE IN HOURS AT 18°-20°C	
1	0	UNFERTILIZED	7	5¾	32 CELL	13	33	NEURAL PLATE
2	1	GRAY CRESCENT	8	8¼	MID - CLEAVAGE	14	44	NEURAL FOLDS
3	2¼	TWO-CELL	9	9¼	LATE CLEAVAGE	15	48	ROTATION
4	3	FOUR-CELL	10	18	DORSAL LIP	16	66	NEURAL TUBE
5	3¾	EIGHT - CELL	11	24	MID - GASTRULA	17	72	TAIL BUD
6	4¼	SIXTEEN - CELL	12	29	LATE GASTRULA			

TEXT-FIG. 1

3

STAGE NUMBER			
	AGE IN HOURS AT 18°-20° CENTIGRADE		
		LENGTH IN MILLIMETERS	
18	80	3·5	MUSCULAR RESPONSE
19	92	4·5	HEART BEAT
20	116	5·5	GILL CIRCULATION HATCHING
21	140	7	MOUTH OPEN CORNEA TRANSPARENT
22	168	8	TAIL FIN CIRCULATION

TEXT-FIG. 2

TEXT-FIG. 3

We have provided a table showing the development of skipper-frog eggs and the time taken to reach the different stages as has been done for frogs, particularly *Rana pipiens* and *R. sylvatica* (see Rugh 1948).

The use of mammalian hormones put on the market has thrown considerable light on the reactivity of the female sex glands of frogs. In this connexion, Witschi, Chang and Segal (1955) and Chang and Witschi (1957) pointed out that in frog, the adrenocorticotrophic hormone and cortisone acetate (cortisone) accelerate the action of the luteinizing hormone contained in the threshold dose of homoplastic frog pituitary glands (1 or 2 glands) injected along with those hormones. The latter authors assumed tentatively a catenary reaction as follows : the high estrogen content of the gravid ovary after the action of follicle-stimulating hormone on it, reacts on the pituitary gland which releases luteinizing and adreno-corticotrophic hormones ; the latter acts upon the adrenal cortex which releases the cortical hormone cortisone. The latter augments the action of the luteinizing factor resulting in ovulation. In this chain reaction, cortisone being the end product, probably plays a better role than either estrogen or adrenocorticotrophic hormone. However, they also state that "cortisone does not appear to be an indispensible factor".

We have not only tested the reaction of the skipper-frog to injections of mammalian hormones but also to them in combination with a threshold or subminimal dose of homoplastic pituitary glands. During December, we injected 25 skipper-frogs each with two homoplastic gland dose ; of these only three gave a few ripe eggs after 15 hrs. The others did not spawn or yield eggs on stripping even 24–48 hrs., after the injection. Obviously the dose is a small one sufficient to prime up the ovary and this has been taken as the threshold dose. With three glands, a large number of skipper-frogs gave ripe eggs on stripping.

For every experiment, we have had controls receiving only two glands to see if the gonad becomes reactive to that dosage as the frogs approach the breeding season. So far (early August, 1958) we have not found the subminimal dose bringing about ovulation in the controls. Along with the subminimal dose, we have injected a number of mammalian hormones to study the augmenting action of the latter. The test animals were injected mostly the previous evening and were examined the next morning for ripe eggs; both spawning and non-spawning test animals were stripped and the eggs fertilized for testing viability.

The following mammalian hormone preparations have been used individually and also in combination with a threshold dose of pituitary glands as referred to above :

1. Follicle-stimulating hormone (Armour Laboratories : R 377210),
2. Luteinizing hormone (Armour Laboratories : R 377279),
3. Human chorionic gonadotrophin (Physex : Dumex; Antuitrin S : Parke, Davis),
4. Pregnant mare serum (Antex : Dumex),
5. Estrogen (Estradiol monobenzoate—Ovocyclin M : Ciba; Estradiol dipropionate—Ovocyclin P : Ciba; Stilbestrol : Boots),
6. Androgen (Methyl testosterone : Boots; Perandren linguets : Ciba),
7. Progesterone (Ethisterone : Boots; Proluton : Schering),
8. Adrenocorticotrophic hormone (Dumex ; Adrenomone : Armour Laboratories),
9. Desoxycorticosterone acetate (Primocort : Schering).
10. Cortisone acetate (Corlin : Glaxo),
11. Thyroid-stimulating hormone (Armour Laboratories : PRR 3-128-92),
12. Growth hormone (Somar : Armour Laboratories : M 108),
13. Thyroxine (Eltroxin : Boots),
14. Insulin (Boots)
15. Oxytocin (Pitocine : Parke, Davis).

It is generally known that mammalian pituitary and chorionic gonadotrophins do not cause frog to yield ripe eggs. There are, however, records where a few species of frogs and toads have reacted favourably, *e.g.*, *Bufo fowleri*, *Rana catesbeiana* (Rugh (1935), *Hyla aurea* (Creaser and Gorbman 1935), *Rana temporaria* (Bellerby 1933 ; Cunningham and Smart 1934 ; Gallien 1937) and *Xenopus laevis* (Hogben, Charles and Slome 1931 ; Bellerby 1933 ; Cunningham and Smart 1934 ; Shapiro and Zwarenstein 1934 ; Shapiro 1935) either to mammalian pituitary or chorionic gonadotrophins. Barth (1933), Creaser and Gorbman (1935) and Rugh (1935) have reported failure to induce *Rana pipiens* to ovulate using sheep and beef pituitary extracts or pregnant mare serum or human pregnancy urine; however, Wright and Hisaw (1946) have succeeded to ovulate *pipiens* with mammalian pituitary gonadotrophins.

Gravid skipper-frogs were injected in the evening a 5 mg. dose of follicle-stimulating hormone (Armour) in distilled water along with a threshold dose of two pituitary glands. Forty per cent of the test animals had spawned by the next morning and also yielded viable eggs on stripping. An equal number of controls receiving 5 mg. dose of follicle-stimulating hormone did not show any reaction. When catfish (*Heteropneustes*) were injected with the same follicle-stimulating hormone, the fish spawned and this naturally led us to infer that the hormone was contaminated with the luteinizing one. On inquiry, Dr. I Bunding (Armour Laboratories) informed us that the follicle-stimulating hormone contained 5 per cent or even more of the luteinizing hormone in it. While the quantity of luteinizing hormone present in the dose was sufficient to spawn the catfish, it was ineffective on frog. But as noted above when the follicle-stimulating hormone was injected along with a threshold pituitary gland dose into skipper-frogs, they yielded viable eggs. During November, skipper-frogs received as much as 10 mg. dose each of the luteinizing hormone and they did not respond. During January, a 5 mg. dose of the hormone in combination with a threshold pituitary gland dose brought about profuse spawning in 90 per cent of the test animals ; the controls receiving two pituitary glands each or 5 mg. dose each of luteinizing hormone alone did not react.

Using chorionic gonadotrophin (Physex : Dumex; Antuitrin S : Parke, Davis), we noticed that even as large a dose as 50 I.U., considering the weight of the frog, could not make the skipper-frogs react. However, when 50 I.U.. of Physex or 25 I.U., of Antuitrin S was used in combination with a threshold pituitary gland dose, 50 per cent ovulation was obtained with the former combination while in the latter, 70 per cent of the test animals spawned. In this connexion, Rugh (1935) reported successful ovulation by injecting human pregnancy urine extract into *Bufo fowleri* and *Rana catesbeiana* and several workers have successfully spawned *Xenopus* with human pregnancy urine.

When a dose of 30 I.U., of pregnant mare serum (Antex : Dumex) was injected into the skipper-frog, they did not react ; however, when the same dosage was given in combination with a subminimal pituitary gland dose, 70 per cent of the test animals spawned. We have replicated this experiment in September 1958 using 75 I.U., of pregnant mare serum (Antex) with half-a-gland as the subminimal dose. The skipper-frogs yielded viable eggs while the controls receiving only half-a-gland of the pituitary could not be stripped. Pregnant mare serum (Serum gonadotrophin) is largely a follicle-stimulating hormone (Dumex firm describe 'Antex' as a highly purified gonadotrophic hormone) and it must have activated the luteinizing pituitary gonadotrophin. However, it is interesting to note in this connexion that catfish do not yield ripe eggs on receiving the pregnant mare serum with the respective threshold pituitary gland dose. Creaser and Gorbman (1935) reported that an extract of pregnant mare serum brought about ovulation in *Hyla aurea* and it is difficult to explain why the hormone did not act on the skipper-frog.

It has been reported that some anurans react to progesterone and male sex hormone. Androgen and progesterone have induced *Xenopus laevis* to spawn (Shapiro 1936) ; Langan (1941) caused ovulation in *Rana pipiens* with testosterone and progesterone but not with estradiol. The reaction of the skipper-frog to estrogen has been very poor, probably due to the medium in which the hormone was injected. When 2.5 mg. of estradiol monobenzoate (Ovocyclin M : Ciba) or estradiol dipropionate (Ovocyclin P : Ciba) was injected after a subminimal pituitary gland dose into the skipper-frog, spawning was noticed in 1 out of ten test animals. Controls receiving an equal quantity of Ovocyclin M or Ovocyclin P respectively did not react. Stilbestrol (Boots) alone in 0.5 mg. doses did not elicit any response in the skipper-frog. It is reported that even large doses (10 mg.) of estradiol have not been able to spawn *Xenopus* (Shapiro 1936) and Langan (1941) noted that *Rana pipiens* did not ovulate when injected with α-estradiol dipropionate.

During February, when a 2 mg. dose of methyl testosterone (Boots) was injected into the skipper-frog, all the test animals spawned. However, when 1 mg. dose of methyl testosterone (Perandren linguets : Ciba) was injected along with the threshold pituitary gland dose, there was comparatively poor spawning and the controls receiving only 1 mg. of the androgen each did not react.

Progesterone also worked very much like the androgen. Skipper-frogs receiving 1 mg. progesterone (Luteostab : Boots) after the subminimal pituitary gland dose, spawned profusely during February; the controls receiving the same dose of progesterone alone could also be stripped for ripe eggs. However, when 1 mg. dose of Ethisterone (Boots) was injected, the frogs did not react. The skipper-frog, therefore, resembles *Xenopus* and *Rana pipiens* in its reaction to androgen and progesterone.

Cortisone acetate (Corlin : Glaxo) has not been able to spawn or ovulate the skipper-frog. Chang and Witschi (1957) also reported that *Rana pipiens* could not be ovulated with cortisone only. Shapiro (1936), however, reported that *Xenopus laevis* ovulated with adrenal-cortical extract. In an experiment with skipper-frogs, cortisone injected with the threshold pituitary gland dose gave 100 per cent success, while the controls receiving only two pituitary gland injections each, one or two yielded a few viable eggs on stripping. On the other hand, a 2.5 mg. dose of desoxycorticosterone acetate (Primocort : Schering) brought about spawning in the skipper-frog ; this result is not unexpected as this cortical steroid resembles chemically progesterone.

We have also computed the time taken for the frogs to spawn after receiving the combination doses. It has already been noted that during March–April when the day temperature reached 27°–28°C, the skipper-frog females receiving 4 pituitary gland injections spawned profusely after the lapse of $9\frac{1}{2}$–10 hours. Even when injected in the evening, the skipper-frog takes the same time to spawn during the night. When three pituitary glands are injected, about 40 per cent of the test females spawn after the lapse of 10 hours. In order to see if in our frogs also, the mammalian hormones have an augmenting effect as described by Chang and Witschi (1957), we injected a combination of 3 pituitary glands with 25 I.U., of chorionic gonadotrophin (Antuitrin S : Parke, Davis) into each of one set, and the other set received 3 homoplastic pituitary glands with 12.5 mg. ($\frac{1}{2}$ ml.) of cortisone acetate (Glaxo) each. At the lapse of 7 hours, all those receiving the chorionic gonadotrophin combination spawned ; fertilized eggs hatched out into tadpoles. Approximately 200 eggs were collected from each skipper-frog and more than 50 per cent of them hatched out; again those frogs started spawning profusely 2–3 hours, after the first stripping. All the cortisone combination injected ones spawned after the lapse of 8 hours, and the eggs were found viable; nearly 70 per cent of the eggs hatched out. As before, these frogs started spawning again 2–3 hours after the first stripping. The controls which received 3 pituitary glands only gave viable eggs after 10 hours ; 10 per cent of these test animals could not be stripped and of

those stripped, only 45 per cent of the eggs hatched out into tadpoles. It is clear from the above that the skipper-frogs receiving combination doses as above not only ovulate 2-3 hours earlier than those receiving pituitary glands but that all the test animals yield ripe eggs.

Unlike cortisone, 5 I.U., of an aqueous solution of adrenocorticotrophic hormone (Corticotropin : Dumex) caused only 25 per cent of the skipper-frogs to ovulate when injected with a threshold pituitary gland dose. However, when 25 U.S.P. units of the hormone suspended in gelatine (Andrenomone: Armour Laboratories) was injected in combination with a subminimal pituitary gland dose, 80 per cent of the test animals spawned indicating that it is not as effective as cortisone. Like cortisone, adrenocorticotrophic hormone by itself has no effect on the skipper-frog ovary.

Growth hormone, so far as we are aware, has not been known to augment the action of the pituitary luteinizing hormone. When skipperfrogs were injected with 1.25 mg. of growth hormone (Somar : Armour Laboratories) in combination with a threshold pituitary gland dose, they spawned profusely and yielded viable eggs. We tested indirectly if the growth hormone contained any ovulating factor in it. Digesting the skipper-frog pituitary glands with ptyalin and trypsin respectively, it was possible to inactivate or destroy the follicle stimulating hormone or the luteinizing one as was done by us for catfish (Ramaswami and Sundararaj 1958). Combining a quantity of the homogenate equal to two pituitary glands of the skipper-frog with 2.5 mg. of growth hormone, the reaction of the test animals receiving the above was studied ; controls received only the homogenate in an equal quantity as the test animals. Test animals receiving ptyalin digested homogenate in combination with growth hormone spawned profusely; the controls receiving only the homogenate also spawned a few eggs ; the spawning caused in the control must be due to the individual action of thel utenizing factor of the skipper-frog's pituitary gland, while the profuse spawning of the experiments must be due the augmenting action of the growth hormone. In the set receiving trypsin digested pituitary gland, the skipper-frogs did not spawn as the luteinizing factor was inactivated or destroyed. If the growth hormone had any appreciable ovulating factor in it, probably the follicle-stimulating hormone would have augmented the action of the ovulating factor. We have also tested using large doses of growth hormone alone so that the ovulating factor, if any, may become effective and bring about ovulation. Skipper-frogs receiving as large a dose as 2.5 mg. of the growth hormone did not react. As the quantity of the growth hormone was limited with us, we could not proceed further with these experiments.

Like growth hormone, the thyroid-stimulating hormone (Armour Laboratories) also augments the activity of the pituitary gonadotrophins. When a dose of 1.25 mg. of thyroid-stimulating hormone was combined with a threshold pituitary gland dose and injected into skipper-frog, 50 per cent of the test animals spawned and gave viable eggs and it is difficult to say if pure thyroid-stimulating hormone alone can act as an activator as the thyroid-stimulating hormone preparations usually contain luteinizing hormone as an impurity. So far as we are aware, there is no record of using the growth and thyroid-stimulating hormones as augmenting agents to activate anuran gonads.

We used synthetic thyroxine (Eltroxin : Glaxo) to find out if like cortisone, this also helped in ripening the eggs. This aspect of the study is still incomplete. As these experiments were conducted during the breeding season of the skipper-frog, it was necessary to find out the subminimal pituitary gland dose necessary for priming up the ovary but not bringing about ovulation. It was found that any dose more than half-a-gland of homoplastic pituitary brought about the ripening of eggs of the skipper-frog, half-a-gland was taken as the threshold pituitary gland dose during the spawning season. When thyroxine was used in

combination with this threshold pituitary gland dose, the skipper frog yielded viable eggs; the hormone by itself had no effect in ripening the eggs of the ovary. Very small doses of the mammalian hormone were found to be effective.

Insulin (Boots) also brings about ovulation in the skipper-frog when used in combination with the subminimal pituitary gland dose, and the hormone by itself has no effect on the ovary.

Oxytocin (Pitocin : Parke, Davis) appears to have no effect on the skipper-frog's ovary even when combined with the threshold pituitary gland dose.

We have not tried any *in vitro* experiments to study the relative speeds of ovulation of gravid frog's ovary as has been done by Wright (1945) or by Chang and Witschi (1957). We feel that the *in vitro* experiments do not simulate *in vivo* ones; they are physiologically very different. An excised piece of ovary put in a hormone medium is physiologically not the same as the ovary of an intact animal receiving a hormone injection and therefore, the two results may not be comparable.

REFERENCES

Adams, A. E. (1931).   Induction of ovulation in frogs and toads *Proc. Soc. Exp. Biol. Med.*, **28**, 677-681.
Atz, J. W. (1957). (See under Pickford and Atz 1957).
Barth, L. G. (1933).   The use of pituitary implants and extracts for obtaining amphibian eggs out of season. *Collecting Net*, **8**, 7-8.
Bellerby, C. W. (1933). The endocrine factors concerned in the control of the ovarian cycle. I, *Xenopus laevis* as test animals. II. *Rana temporaria* as test animal. *Biochem. Jour.*, **27**, 615-620, 2022-2030.
Burrows, H. (1945). Biological Actions of Sex Hormones. Cambridge.
Chang, C. Y. and Witschi, E. (1957). Cortisone effect on Ovulation in the frog. *Endocrinol.* **61**, 514-520.
Creaser, C. W. and Gorbman, A. (1935). Apparent specificity of the induced ovulation reaction in amphibia. *Am. Jour. Physiol.*, **113**, 32.
——— (1939). Species specificity of the gonadotropic factors in vertebrates. *Quart. Rev. Biol.*, **14**, 311-331.
Cunningham, J. T. and Smart, W. A. M. (1934). The structure and origin of corpora lutea in some of the lower vertebrata. *Proc. roy. Soc., London*, **116B**, 258-281.
Gallien, L. (1937). Action comparée des extraits hypophysaires et des substances gonadotropes de l'urine sur l'ovulation chex *Rana temporaria* L. *Compt. rend. Soc. de Biol.*, **124**, 874-877.
Hogben, L., Charles, E. and Slome, D. (1931). Studies on the pituitary. VIII. The relation of the pituitary gland to calcium metabolism and ovarian function in *Xenopus. Jour. Exp. Biol.*, **8**, 345-354.
Langan, W. B. (1941). Ovulatory response of *Rana pipiens* to mammalian gonadotropic factors and sex hormones. *Proc. Soc. Exp. Biol. Med.*, **47**, 59.
McCann, C. (1932). Notes on Indian batrachians. *J. Bombay Nat. Hist. Soc.*, **36**, 152-180.
Pickford, G. E. and Atz, J. W. (1957). The Physiology of the Pituitary gland of Fishes. New York Zoological Society, New York.
Ramaswami, L. S. and Lakshman, A. B. (1958a). Ovulation induced in frog with mammalian hormones. *Nature*, London, **181**, 1210.
——— (1958b). Spawning catfish with mammalian hormones. *Nature*, London, **182**, 122-123.
Ramaswami, L. S. and Sundararaj, B. I. (1956). Induced spawning in the Indian catfish. *Science*, **123**, 1080. 1956.
——— (1957a). Some aspects of induced spawning in the catfish *Heteropneustes. Naturwissenchaften*, **2**, 46.

Ramaswami. L. S. and Sundararaj, B. I. (1957b). Inducing spawning in the Indian catfish *Heteropneustes* with pituitary injections. *Acta Anat.*, **31**, 551–562.

———— (1957c). Induced spawning in the catfish *Heteropneustes* with mammalian chorionic gonadotrophins. *Ibid.*, **32**, 236–239.

———— (1957d). Induced spawning in the catfish *Clarias*. *Naturwissenschaften.*, **13**, 384.

———— (1958). Action of enzymes on the gonadotrophic activity of pituitary extracts on the Indian catfish *Heteropneustes*. *Acta Endocrinol.*, **27**, 253–256.

Rugh, R. (1935). Ovulation in the frog. I. Pituitary relations in induced ovulation. II. Follicular rupture to fertilization. *Jour. exp. Biol.*, **81**, 149–193.

———— (1948). Experimental Embryology. Minnesota.

Shapiro, H. A. (1936). Experimental induction of coupling in *Xenopus laevis* with production of fertilized eggs. *Nature*, London, **135**, 510.

Shapiro, H. A. and Zwarenstein, H. (1934). A rapid test for pregnancy on *Xenopus laevis*. *Ibid.*, **133**, 339 and 762.

Stroganov, N. S. and Alpatov, V. V. (1951). A new unit for determining the activity of the hypophysis in fish. *Rybnoe Khoziaaistvo*, **27**, (9), 56–60. (quoted in Pickford and Atz 1957).

Turner, C. D. (1955). General Endocrinology. Philadelphia.

Wills, I. A., Riley, G. M. and Stubbs, E. M. (1933). Further experiments on the induction of ovulation of toads. *Proc. Soc. exp. Biol. Med.*, **30**, 784–786.

Witschi, E., Chang, C. Y. and Segal, S. J. (1955). On the hormonal control of ovulation and spermiation in Amphibians. *Anat. Rec.*, **122** 452.

Wright, P. A. (1945). Factors affecting in vitro ovulation in frogs. *Jour. exp. Zool.*, **100**, 565.

Wright, P. A. and Hisaw, F. L. (1946). Effect of mammalian pituitary gonadotropins on ovulation in the frog *Rana pipiens*. *Endocrinol.*, **39**, 247.

OVIPOSITION BEHAVIOUR OF *DIADROMUS (THYRAELLA) COLLARIS* GRAVENHORST (ICHNEUMONIDAE : HYM.), A PARASITE OF CABBAGE DIAMOND-BACK MOTH, *PLUTELLA MACULIPENNIS* CURTIS (TINEIDAE : LEP.)

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ABSTRACT

Diadromus collaris Grav. wasps kept at 18°C laid more eggs and lived longer than those kept at 25°C. The females when offered unparasitized hosts distributed their eggs selectively. Inspite of this some of the pupae were superparasitized, whereas others were unparasitized. Thus the parasites, throughout their life, distributed eggs selectively in a proportion of the host pupae offered.

Whereas the production of mature eggs in the female is in some way stimulated by the presence of hosts (Lloyd, 1940), the present studies reveal that the number of hosts available has no influence on the total number of eggs produced by the parasite.

INTRODUCTION

Diadromus (Thyraella) collaris Grav. is one of the important parasites of the diamond-back moth, *Plutella maculipennis* Curtis, which attacks its host in the prepupal and pupal stages. Lloyd (1940) studied its oviposition behaviour in the laboratory and found that it did not lay eggs in hosts that had already been parasitized for more than a day by the same species or another one. However, when more recently parasitized hosts were given, the parasite females often laid eggs in the same host even though other unparasitized hosts were present in their vicinity. It indicated that superparasitism is an inherent weakness of the parasite. However, if no hosts were provided for some time, the females stopped producing eggs as no mature eggs were found in the oviduct.

The present studies pertain to the oviposition behaviour of parasites provided with 4 and 8 host pupae per day throughout the length of their life. Since the females were encaged in a limited space in which they were bound to come across the hosts offered and the number of eggs laid by each female was recorded every day for the whole duration of its life, it was easy to study the number of eggs produced from day to day and thus evaluate the degree of parasitism among the two constant number of hosts available each day at the two constant temperatures, viz., 18°C and 25°C.

MATERIALS AND METHODS

The parasites used in these experiments were reared in the laboratory at 18°C and 25°C. On emergence the males and females were kept together for one day so as to ensure that all the females had mated before being used for experiments.
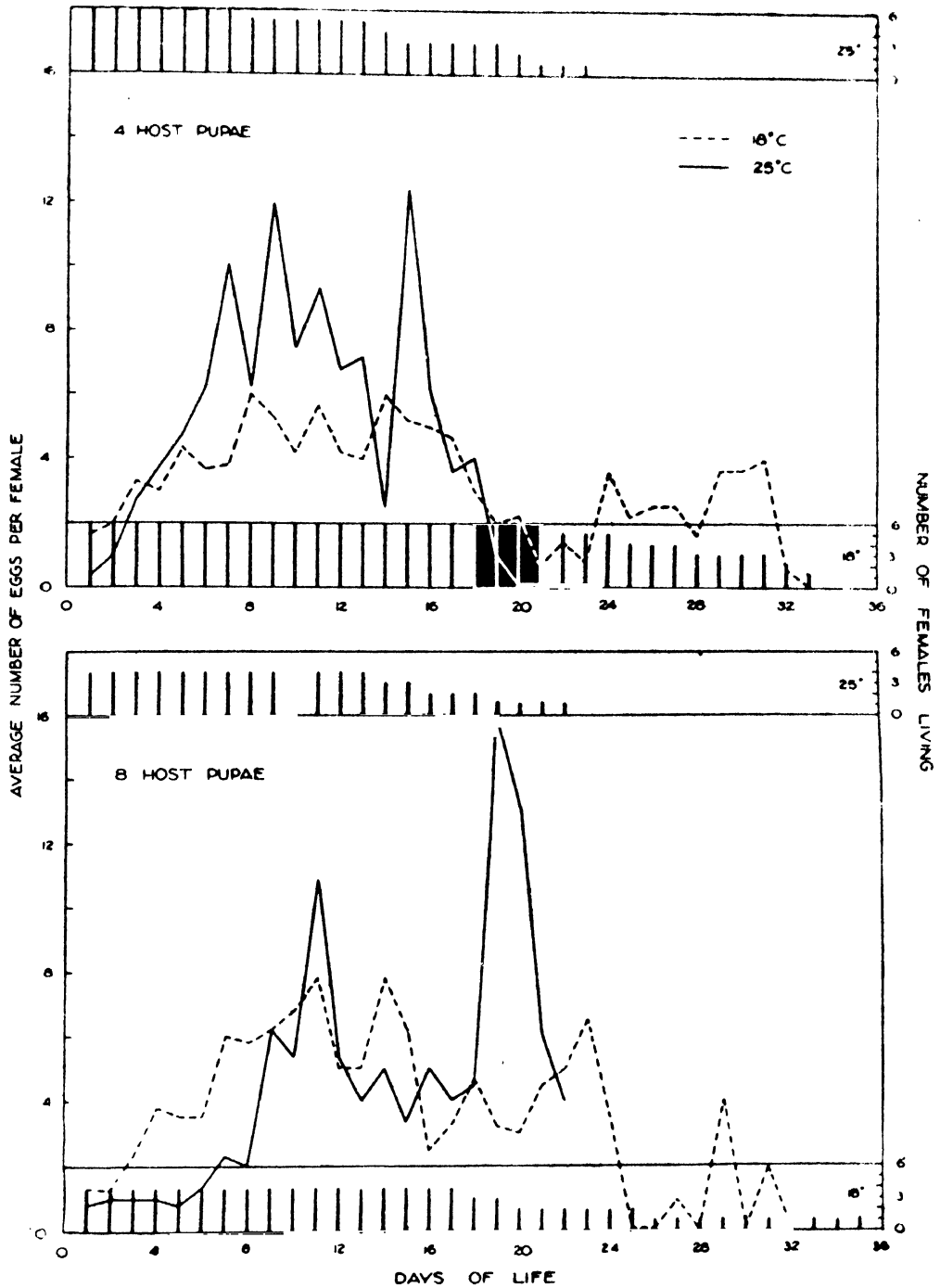
TEXT-FIG. 1

The curves show the daily production of eggs by *Diadromus*. The histograms show
the survival rates of the egg-laying females.

Pairs of males and females were then transferred to glass specimen tubes 5 inches long and 1¼ inches in diameter. The mouth of the tube was closed by a piece of muslin cloth with a split raisin attached on the inside. Of the ten females at each of the above temperatures, six were offered four and the remaining four, eight host pupae per day. The host pupae were reared in the laboratory. Small pieces of leaf were cut around the pupae thereby not disturbing them from their place of attachment : if the silken cocoon of the pupae is damaged the host is not accepted by the parasite. The pupae were arranged along the tube kept horizontal to the floor. As the acceptibility of a pupa is determined by its age (Lloyd, 1940) all the hosts offered to the experimental females were of 12 to 24 hours of age. The pupae offered to the experimental parasites were replaced by new ones every twenty four hours. The pupae were dissected under a binocular microscope and the eggs in each one of them were recorded day by day.

EXPERIMENTAL RESULTS

The mean life of a female at 18°C was 26.0 days as compared to 16.5 at 25°C and the difference was significant at the 1 per cent level (Table I). The number of eggs laid at 18°C was 96.5 and at 25°C was 74.9 (significant at 5 percent level—Table I). There was no significant influence of the number of hosts provided on the number of eggs laid by a parasite or on its length of life. The females started to lay eggs soon after mating and continued to lay at least some eggs each day until they died. The day to day variation in the number of eggs laid was greater at 25°C than at 18°C (Fig. 1). There is a slight increase in the number of eggs laid during the later part of life (Fig. 1) which may possibly be due to the fact that those females that lived longer than the others also produced a greater number of eggs.

TABLE I

Mean Length of Life and the Number of Eggs per Female

Host Pupae Per Female Per Day

Temperature	8 pupae (Mean of 4 females)		4 pupae (Mean of 6 females)	
	Life (days)	Eggs in life-time	Life (days)	Eggs in life-time
25°C	17.0	64.5	16.0	85.2
18°C	24.0	96.8	28.0	96.3
Standard deviation ±	2.62	19.5	2.62	19.5

Degrees of Freedom :   16

The effect of temperature on the length of life was significant at the 1 per cent level and that on the number of eggs laid was significant at the 5 per cent level. None of the other effects or their interactions was significant.

The oviposition behaviour of the females was studied from the daily record of egg-laying. Ignoring the temperature, the difference between females and their age, the data were pooled into different groups representing the number of eggs laid per female per day, separate for four and eight host pupae. When four hosts were offered per day, the χ^2 was highly significant in all the sections

except six eggs (Table II). In the latter case the non-significance was probably due to the fact that the selective behaviour pattern was so similar to the random distribution that the difference could not be detected. However, in all the other sections of the data selective distribution was evident. When eight host pupae were offered per day, χ^2 was non-significant in all sections except four eggs in which case it was significant at the 5 per cent level (Table III). The reason for non-significance may again be the same as above. With eight pupae the expectation for a random choice of pupae comes closer to the expectation for a model in which the females are distributing their eggs at random through a fixed proportion of the pupae and the difference between the two could not be detected. But the significance of the test on four eggs per day is a pointer that probably selective behaviour is still in operation.

<div align="center">

TABLE II

Oviposition behaviour of Diadromus
4 hosts

(Ignoring temperature, differences between females, and age-effects)

</div>

Eggs per female per day	Number of hosts attacked	Observed	Expected	χ^2
2	2	41	33.0	
	1	3	11.0	
	Total	44	44.0	$\chi_c^2 = 6.82$ P < 0.01
3	3	21	12.0	
	2 or 1	11	20.0	
	Total	32	32.0	$\chi_c^2 = 9.63$ P < 0.00
4	4	10	2.5	
	3	13	14.6	
	2 or 1	3	8.9	
	Total	26	26.0	$\chi_2^2 = 27.55$ P < 0.001
5	4	13	5.4	
	3	3	13.5	
	2 or 1	7	4.1	
	Total	23	23.0	$\chi_2^2 = 20.88$ P < 0.001
6	4	7	6.1	
	3	6	8.4	
	2 or 1	3	1.5	
	Total	16	16.0	$\chi_2^2 = 2.43$ non. sig.

The lack of slope in the curves of Figure 2 indicates that the number of hosts attacked is not dependent on the number of eggs laid per day. The contingency χ^2 test of the data on four pupae also confirmed that the selective behaviour was not dependent on the number of eggs laid on a particular day.

<div align="center">

TABLE III

Oviposition behaviour of Diadromus

8 hosts

(Ignoring temperature, differences between females, and age effects)

</div>

Eggs per female per day	Number of hosts attacked	Observed	Expected	χ^2
2	2	21	18.4	
	1	0	2.6	
	Total	21	21.0	$\chi^2_c = 2.99$ non. sig.
3	3	10	9.2	
	1 or 2	4	4.8	
	Total	14	14.0	$\chi^2_c = 0.20$ non. sig.
4	4	9	4.9	
	3, 2, or 1	3	7.1	
	Total	12	12.0	$\chi^2_3 = 4.42$ P < 0.05
			2.5	
	4	5	6.1	
	3, 2, or 1	2	3.4	
	Total	12	12.0	$\chi^2_3 = 3.40$ P < 0.20
	6 or 5	6	6.5	
	4	6	5.8	
	3, 2, or 1	2	1.7	
	Total	14	14.0	$\chi^2_3 = 0.03$ non. sig.

There are big differences between the positions of the curves for four and eight host pupae (Fig. 2 and 3) which means the wasps show greater dispersion of eggs with a larger number of hosts available. Thus all the evidence, namely the big differences in the positions of the curves for four and eight pupae in Fig. 2, the non-significance of χ^2 for eight pupae compared to the significant χ^2 for four pupae and the lack of slope in the curves in Fig. 2, seem to point to the tentative conclusion that the females were attracted to lay eggs into some

pupae but not others. The experimental females were not offered such hosts as appeared abnormal, so one cannot tell by what criterion they accepted some and rejecetd others. Since the host pupae were more or less of the same age (12 24 hours), that could not have affected their acceptability to the female. It may be that some such factor as the quality of food eaten by the host which can affect its fecundity and longevity (Atwal, 1955) may also influence its acceptability to the parasite.

OVIPOSITION BEHAVIOUR OF DIADROMUS.
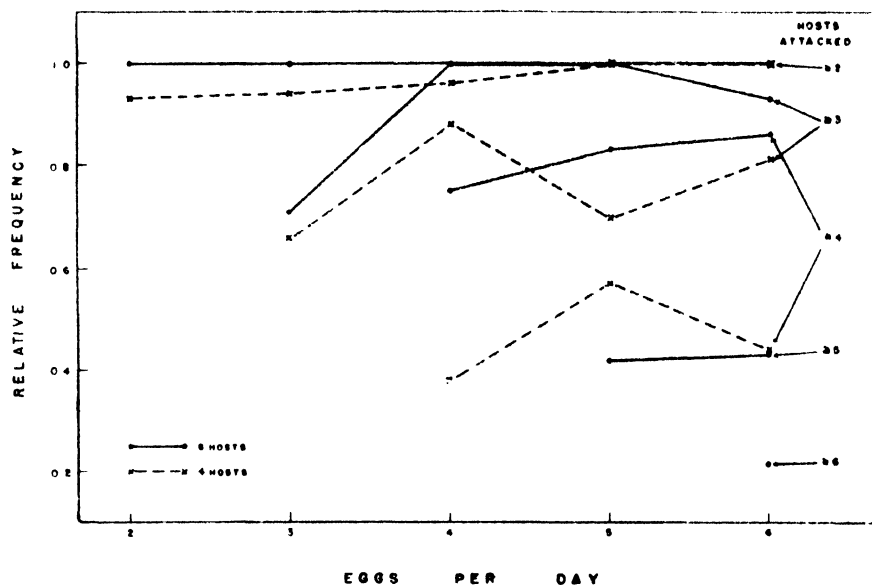
TEXT-FIG. 2

The individual curves relate to the occasions on which 2 or more, 3 or more, 4 or more, 5 or more and 6 or more pupae were attacked. The relative frequencies may be calculated from the numbers in tables II and III. For example, with four pupae exposed there were 26 occasions on which 4 eggs were laid in a day. Of these 26 occasions there were 23 when the 4 eggs were distributed through 3 or more pupae

giving a relative frequency of $\frac{23}{26}$ or 0.88.

Diadromus wasps, which can distinguish previously parasitized hosts from the unparasitized ones (Lloyd, 1940) when given a choice of variable number of unparasitized hosts seem to distribute eggs selectively upto a certain extent. Due to some unknown cause it rejects a certain proportion of the unparasitized hosts provided. Probably it can differentiate the unacceptable pupae through some peculiar sense of its own. There is no way to distinguish from the data the rejected from the accepted pupae so that oviposition behaviour just in the latter group cannot be analysed. Thus a complicated hypothesis might be that the female parasite tends to distribute its eggs evenly through a certain proportion of the pupae. This behaviour of the female is constant throughout the entire life. Since the female lays the same number of eggs whether offered 4 or 8 host pupae the number of eggs produced depends on factors other than the number of hosts available. However, Lloyd (1940) found that the female stopped producing eggs if no hosts were provided for some time. Thus the host provides

stimulus for the female in a way that the production of eggs is initiated but once that has happened the actual number of eggs produced is independent of the number of hosts available. As compared to this the temperature at which the female is reared and kept has a significant influence on the total number of eggs produced by it, 18 as compared to 25°C being more favourable.

ACKNOWLEDGEMENTS

REFERENCES

Atwal, A.S. (1955). Influnce of temperature, photoperiod, and food on the speed of development, fecundity and other qualities of the diamond-back moth, *Plutella maculipennis* (Curtis) (Tineidae; Lepidoptera). *Aust. J. Zool.*, **3**, 185-221.
Fisher, R.A., and Yates, F. (1948). Statistical Tables for Biological, Agricultural and Medical Research. (Oliver and Boyd : London).
Lloyd, D.C. (1940). Host selection by hymenopterous parasites of the moth *Plutella maculipennis* Curtis, (Tineidae, Lepidoptera). *Proc. Royal soc. B.* **128** : 451-484.

DISTRIBUTION OF ALKALINE PHOSPHATASE DURING THE EARLY DEVELOPMENT OF *PILA VIRENS* (LAMARCK)

by K. RAMAMOORTHI, *Department of Zoology, Annamalai University, Annamalainagar*

(Communicated by B. R. Seshachar, F.N.I.)

ABSTRACT

Phosphatase activity is seen only in the periphery of the full grown oocyte. In the fertilised egg the enzyme is concentrated in the vegetal region. A weak phosphatase activity during cleavage stages and a high phosphatase activity in the endoderm and mesoderm cells during gastrulation is observed. The shell-gland in the early veliger shows high phosphatase activity.

INTRODUCTION

Investigations on alkaline phosphatase activity in animals have so far been mostly with reference to adult tissues. Studies on the alkaline phosphatase activity in embryonic development have been relatively few. Krugelis (1947), Wicklund (1948), Gustafson and Hasselberg (1950), and Mazia *et al.* (1948) studied the alkaline phosphatase distribution in the developmental stages of the sea urchin. Fitzgerald (1949) studied the alkaline phosphatase activity in the developing grasshopper egg. Moog (1944) studied the localisation of alkaline phosphatase and acid phosphatase in the early embryogenesis of chick. Yao (1950*a*, *b*) and Brachet (1946) studied the localisation in the embryonic and post-embryonic development of *Drosophila melanogaster* and in the early development of amphibia respectively. The present study relates to the localisation of alkaline phosphatase during the early stages of development of *Pila virens*, the chemical embryology of which has been under investigation.

MATERIAL AND METHODS

For the detection of alkaline phosphatase Gomori's (1949) technique was employed with the precautions suggested by Danielli (1946). Embryos were fixed in chilled acetone for 8 hours dehydrated in absolute alcohol and benzene and infiltrated at 56°C. Sections were cut at 6 to 8 μ thick and after removing the paraffin, sections were brought down to water and incubated at 37°C in a substrate medium containing glycerophosphate at pH 9.4 and were later treated with cobalt chloride and ammonium sulphide. Following the hydrolysis of the ester by the tissue enzyme a black deposit of cobalt sulphide was observed at sites of enzymatic activity. Controls were run as a routine procedure and these showed no sign of reaction.

OBSERVATIONS

1) In the full grown oocyte the site of phosphatase activity is indicated by scattered granules in the peripheral region. In the fertilised egg the distribution of the enzyme is in the form of numerous granules more or less evenly distributed throughout the cytoplasm excepting near the vegetal pole. In this region there is a dense accumulation of granules showing intense phosphatase activity (Fig. 1.)

(2) During the cleavage stages a diminution in phosphatase activity is indicated by the scattered distribution of granules in the cells.

(3) During gastrulation, however, intense phosphatase activity is noticed. The endoderm cells and the mesoderm cells show a high degree of phosphatase activity in the cytoplasm (Fig. 3). In the ectoderm cells the nucleus alone takes a feeble stain and there is no indication of any activity of the enzyme in the cytoplasm of these cells.

(4) In the early veliger the region of the shell-gland, which is at this stage in the form of an ectodermal invagination, forms the site of high phosphatase activity.

DISCUSSION

In the embryo of the grasshopper, according to Fitzgerald (1949), as stated already, there is no detectable alkaline phosphatase activity until late in development, and it then lasts till just before hatching. In the unincubated stage of the blastoderm of the hen's egg alkaline phosphatase is indiscernible, and also in all embryonic tissues during the first two or three days of development (Moog, 1944). Phosphatase persists as long as a tissue remains undifferentiated, and as differentiation proceeds, phosphatase in some tissues disappears, and in others accumulates in a higher concentration. In the sea urchin eggs Gustafson and Hasselberg (1950) noted a constant and weak activity during cleavage stages and a rapid rise of the activity after the appearance of primary mesenchyme cells in the blastocoele. In the present study on the embryos of *Pila* a weak and constant activity during cleavage stages and rise during gastrulation has been observed and this seems to be in agreement with what Gustafson and Hasselberg (1950) and Mazia *et al.* (1948) observed in sea urchin eggs.

The interesting correlation between phosphatase activity and growth and differentiation was first demonstrated in chick embryogenesis by Moog (1944). Similar evidence has been presented in the early development of amphibia by Brachet (1946) and in the sea urchin by Mazia *et al.* (1948). The findings of the several authors generally tend to show that in embryonic tissue the activity is weak during the early stages of development but becomes intense at the beginning of differentiation.

Studies made by the author on RNA distribution in the early development of *Pila* (*unpublished*) show that high phosphatase activity during gastrulation is associated with high content of ribonucleic acid which is known to be closely associated with protein synthesis.

That alkaline phosphatase activity plays an important part in the process of calcification has been shown by Wagge (1951), Manigault (1939) and Bourne (1943). Manigault (1939) has in fact demonstrated biochemically that during regeneration of the shell in *Helix* there is an increase in the amount of alkaline phosphatase in the mantle, in the extracts of digestive gland and in the blood. Wagge (1951) has shown that alkaline phosphatase is abundant during shell repair especially around digestive gland lime cells. Bourne (1943) has observed a high phosphatase activity in his histochemical studies on shell gland. In the present study it is seen that alkaline phosphatase activity is associated with the embryonic shell gland.
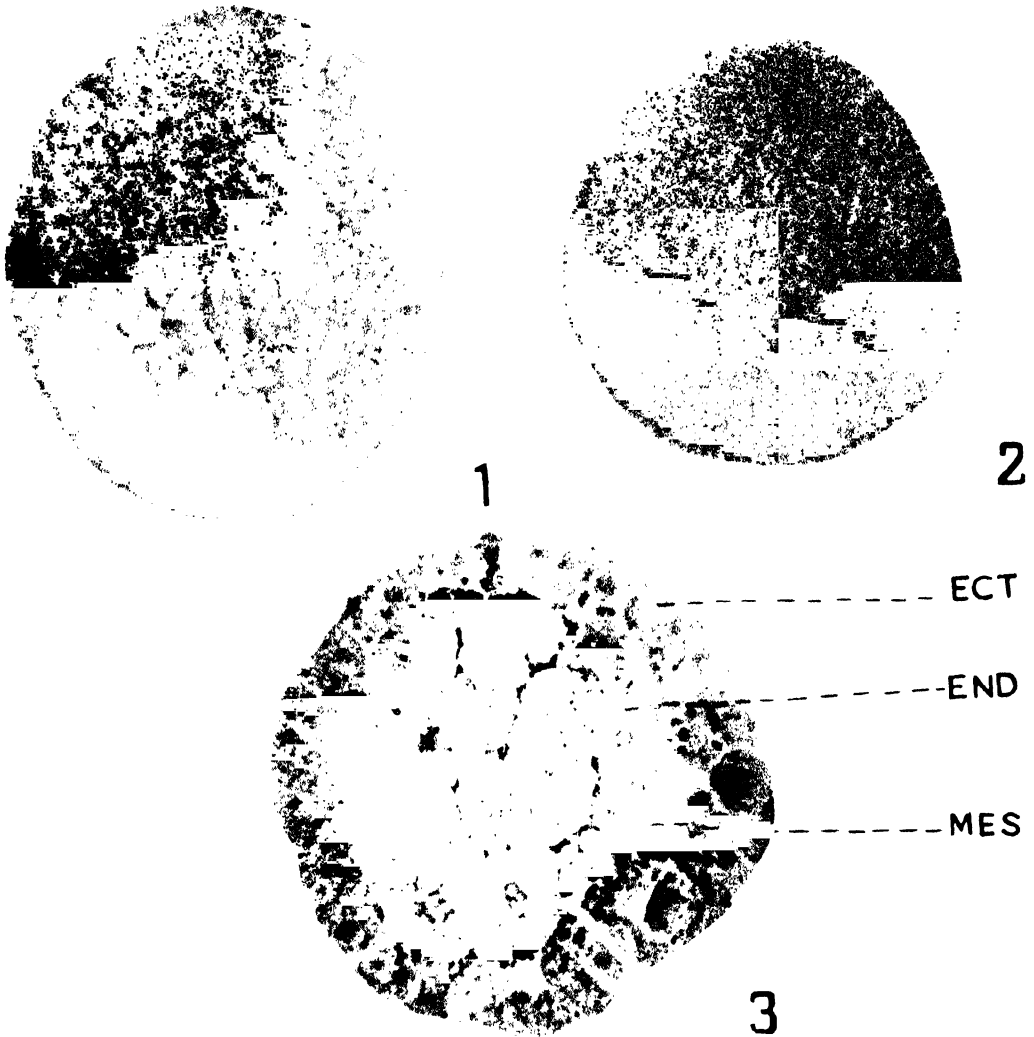
ACKNOWLEDGEMENTS

Fig. 1. Photomicrograph showing the distribution of alkaline phosphatase activity in the fertilised egg. × Ca 1000.

Fig. 2. Photomicrograph showing the control of the above stage. × Ca 800.

Fig. 3. Photomicrograph indicating the localisation of alkaline phosphatase in the gastrula stage. Ca 1000.

(ECT-Ectoderm ; END-Endoderm ; MES-Mesoderm)

BIBLIOGRAPHY

Brachet, J. (1946). *Experientia*, **2**, 143.

Bourne, G. H. (1943). The distribution of alkaline phosphatase in various tissues. *Quart. J. Exp. Physiol.*, **32**, 1–17.

Danielli, J. F. (1946). A critical study of the techniques for the localisation of alkaline phosphatase. *J. Expl. Biol.*, **22**, 110.

Fitzgerald, L. R. (1949). The alkaline phosphatase of the developing grasshopper egg. *J. exp. Zool.*, **110**, 461–487.

Gomori, G. (1949). Histochemical specifity of phosphatase in tissue sections. *Proc. Soc. Exp. Biol. Med.*, **70**, 7–11.

Gustafson, T., and Hasselberg, I. (1950). Alkaline phosphatase activity in the developing sea urchin eggs. *Exptl. Cell Res.*, **1**, (3), 371–75.

Krugelis, E. J. (1947). Alkaline phosphatase in the early development of *Arbacia punctulata*. *Biol. Bull.*, **9**, (3), 209 Abs.

Manigault, P. (1939). Researches sur le calcaire cher les Mollesques phosphatases et precipitation calcique histochemides calcium. *Ann. Inst. Oceanogr.* Paris, N.S., **18**, 331–428.

Mazia, D., Bluementhal, G., and Benson, E. (1948). The activity and distribution of desoxyribonuclease and phosphatases in the early development of *Arbacia punctulata*. *Biol. Bull.*, **95**, (2), 250 (Abs.).

Moog, F. (1944). Localisation of alkaline and acid phosphatases in the early embryogenesis of the chick. *Ibid.*, **86**, 51–80.

Sundaraja Iyengar, V. K. (1954). Studies on the reproductive tract of *Ariophanta maderaspatana*. (Gray) (Mollusca : Pulmonata) Part I. Histochemistry of the amatorial organ. *Proc. Nat. Inst. Sci. India*, **20**, (6), 683–691.

Wagge, L. E. (1951). The activity of amoebocytes and alkaline phosphatase during the regeneration of the shell *Helix aspersa*. *Quart. J. micr. Sci.*, **92**, 302–321.

Wicklund, E. (1948). Distribution of alkaline phosphatase in the eggs of sea urchin. *Nature*, **161**, (4092), 556–557.

Yao, T. (1950a). Cytochemical studies on the embryonic development of *Drosophila melanogaster* II. Alkaline and acid phosphatase. *Quart. J. micr. Sci.*, **91**, (1), 79–88.

——— (1950b). The localisation of alkaline phosphatase during the post-embryonic development of *Drosophila melanogaster*. *Ibid.*, **91**, (1), 89–105.

GENUS *RICCIA* IN INDIA—III*

SPECIES OF *RICCIA* FROM THE EAST HIMALAYAN TERRITORY WITH DESCRIPTION OF A NEW SPECIES, *R. ATTENUATA* PANDÉ *SP. NOV.***

by S. K. PANDÉ *and* RAM UDAR, *Department of Botany, Lucknow University*

(Communicated by G. P. Majumdar. F.N.I.)

ABSTRACT

The East Himalayan Territory (Pandé, 1958) constitutes the area East and South-East of Nepal, i.e., Sikkim, North-West Bengal, Assam, Manipur and Tripura. Extensive collections of Bryophytes from this territory were made by one of us (Pandé) in the region of the Sikkim-Himalayas (Darjeeling-Sandakpu) in the year 1941 and from Khasi-Jayanti Hills (Assam) in the year 1952. The species of *Riccia* from this collection have been described. In this communication the following eleven species of *Riccia*, including a new species, have been dealt with :—

R. frostii Aust., *R. crystallina* L., *R. discolor* L. *et* L., *R. ciliata* Hoffm., *R. glauca* L., *R. huebeneriana* Lindenb,. *R. sorocarpa* Bischoff., *R. gangetica* Ahmad, *R. billiardieri* Mont. *et* N., *R. beyrichiana* Hampe and the new species, *R. attenuata* Pandé (see Stephani, 1900 ; Mitten, 1861 ; Kachroo, 1950, 1952, 1954 ; Udar, 1956 ; Pandé and Udar, 1957 and Pandé and Udar, present communication). The latter has been discovered from the Kamakhya Hills, Assam. It belongs to the *Ricciella* section of the genus and is characterized by thin membranous thallus, with strongly attenuate wings, and with spores having complete reticulations on the outer face and incomplete and irregular reticulations on the inner faces. This species has also been identified from *Pandé collection* from Kandy, Ceylon. The paper includes a discussion for the segregation of this new species and a key for the identification of all the species known from this territory. *R. beyrichiana* Hampe, a common species from America and Europe, has been reported for the first time from India. An illustrated account of this species, based on the Indian specimens, has also been given. *R. kashyapii* Kachroo has been reduced to a synonym of *R. huebeneriana* Lindenb.

INTRODUCTION

From the data available on the study of the hepatic vegetation of India, Pandé (1958) tentatively proposed six Bryogeographical units of the Indian territory with the East Himalayan region constituting the area East and South-East of Nepal, i.e., Sikkim, North-West Bengal, Assam, Manipur and Tripura.

Extensive collections of Bryophytes from the East Himalayan territory were made by one of us (Pandé) on two different occasions. In the region of the Sikkim Himalayas (Darjeeling-Sandakpu) in the year 1941, an area approximately 200 miles was covered in a circular tour ranging in altitude from 4,000 ft. to 12,000 ft. and in the territory of Assam collections were made from Khasi-Jayanti Hills, Gauhati, Shillong, Mauflong virgin forest, Cherrapunji, Dawki and other places in 1952, ranging in altitude from 4,000 to 8,000 ft. These collections, numbering several hundred packets, have now undergone a preliminary sampling and the detailed observation on the species of *Riccia* is being presented in the present communication. A complete regional flora of this territory will follow in due course in a separate paper.

Fortunately, all the species of *Riccia* collected were fertile and thus a complete study could be undertaken. Most of them were also suitably preserved and relevant notes taken in the field.

The earliest undoubted record of a species of *Riccia* from this territory is an unidentified species described and illustrated in the posthumous memoirs of Griffith (1849, 1849a) from the shores of Brahmaputra in Assam. Stephani (1900) apparently cited the specimens of Griffith's collection under the new species, *R. microspora*, instituted by him for a collection from Bengal. As the original specimens of this species from Stephani's Herbarium (Griffith's collection), obtained through the courtesy of Dr. C. E. B. Bonner, Conservatoire, Botanique, Geneve, has unmistakably been found to be identical with *R. frostii* Aust. (see Pandé and Udar, 1957), *R. microspora* was reduced as a synonym of *R. frostii* (Pandé and Udar, 1957). Mitten (1861) reported *R. crystallina* L., *R. ciliata* Hoffm. and *R. discolor* L. et. L., the first two from Bengal and the third from Central Himalaya. Then apparently a long gap intervened before Chopra (1938) listed *R. himalayensis* St. a composite species (Udar, 1957), from Darjeeling based on a collection of Tirrunarayanan in 1917. From this record alone it is rather difficult to ascribe it to any one of its segregates (see Udar, 1957 ; Pandé and Udar, 1957) since all of them occur in this area.

Kachroo (1950,1952) listed *R. discolor* from Gauhati, Shillong, Jorhat (Assam) and *R. frostii* from Gauhati (Assam) and later (Kachroo, 1954) described a new species, *R. kashyapii* Kachroo and reported *R. glauca* L. from Assam.

In a recent study of 'Pandé collection' from Darjeeling in 1941 Udar (1956) reported *R. huebeneriana* and *R. sorocarpa*, the former from Badampton about 7 miles from Darjeeling and the latter from the compound of the Dak bungalow in Phaloot (ca, 12,000 ft.). *R. kashyapii*, earlier described by Kachroo (1954), approaches very near *R. huebeneriana* and the two species occur in the same territory and are identical. The minor differences may only be of the nature of ecological variations.

Thus a perusal of the above publications from the East Himalyan territory would show that 7 species, viz., *R. frostii* Aust., *R. crystallina* L., *R. discolor* L. et L., *R. ciliata* Hoffm., *R. glauca* L., *R. huebeneriana* Lindenb. and *R. sorocarpa* Bischoff. occur in this area. Our study of specimens of *Riccia* from this territory has revealed the presence of four more species, viz., *R. billardieri* Mont. et N. *R. gangetica* Ahmad, *R. beyrichiana* Hampe including *Riccia attenuata* Pandé, a new species, bringing the total to 11 species.

The East Himalayan territory is, however, more extensive than has been covered so far and future search would undoubtedly bring to light several other species.

Since there is some controversy regarding the status of the genus *Ricciocarpus* the authors have deferred the discussion of this plant in the present communication and propose to take it up in a separate paper. A key for the identification of the species of *Riccia*, known from this territory, is given below.

KEY FOR IDENTIFICATION

1. Thallus with compact and narrow air spaces—sub-genus *Euriccia* .... 　3
2. Thallus spongy with wide air spaces—sub-genus *Ricciella*　　.... 　9
*3a. Thallus ciliate, cilia long and curved, spore 80μ　　1. *R. ciliata.*
3b. Thallus non-ciliate　.... 　.... 　.... 　.... 　4
4a. Plants dioecious, spore 80-120μ, reticulate, unwinged, tri-radiate mark inconspicuous　.... 　.... 　2. *R. discolor.*

*Species included in the key on the basis of their report. They are not represented in our collection.

4b.   Plants monoecious   5
5a.   Spore winged (with a distinct perispore)   6
5b.   Spore unwinged (without a perispore)   8
6a.   Thallus 3 times broader than high   7
*6b.   Thallus 4-6 times broader than high, spore 80-100μ, highly papillose
       in profile   ....   3. *R. glauca.*
7a.   Segments sulcate, *sulcus narrow and deep anteriorly, shallow and broad
       behind,* epidermis one-layered   ....   4. *R. beyrichiana.*
7b.   Segments sulcate, sulcus narrow, prominent anteriorly, *epidermis
       2-layered,* upper layer mamillate usually disorganising, lower layer
       thick-walled and persistent   ....   5. *R. sorocarpa.*
8a.   Thallus *about 3 times broader than high, spore opaque turning perfectly
       black at maturity,* reticulate *with 8-16 small reticulations on outer
       face*   ....   6. *R. gangetica.*
8b.   Thallus *about 4-6 times broader than high,* spore reddish brown, 5-7
       reticulations on outer face, *angles of reticulations extended in prominent
       projections* capped by an undulating membrane   7. *R. billardieri.*
9.   Plants dioecious, growing in well defined rosettes, male rosettes usually
       pinkish, spore upto 50μ, tetrahedral with prominent tri-radiate mark,
       incompletely and irregularly reticulate, winged   8. *R. frostii.*
10.   Plants monoecious   11
11a.   Spore *with 4-5 large incomplete reticulations on the outer face*
       ....   9. *R. crystallina.*
11b.   Spore with 5-10 complete reticulations on the outer face   12
12a.   Thallus about 3 times broader than high, older parts pitted due to
       disorganization of the epidermal layer, spore upto 60μ, 5-6 reticulations
       on the outer face, wing entire   ....   10. *R. huebeneriana.*
12b.   Thallus *about 10-15 times broader than high, spore* upto 85μ, 5-7
       reticulations on the outer face, wing crenate   11. *R. attenuata.*

DESCRIPTION

1. *Riccia attenuata* Pandé *sp. nov.*

Herba monoica, lucide viridis, ut plurimum parte parti impendente, saepe
efformans rosulas 5 mm. diam. ; thallus semel vel bis furcatus, 3—6 mm. longus ;
2-3 mm. latus, sulcatus, sulco profundiore ante ; squamae hyalinae, inconspicuae;
rhizoidea simplicia et tuberculata, tuberculis prominentibus ; sectio transversa
10-15-plo latior quam alta, alis alte attenuatis in utroque latere ; cellulae epider-
males semel seriatae, hyalinae, sphaericae ; ostiolum antheridiale ca. 50μ supra
superficiem thalli ; sporae pallide brunneae, 65-85μ ad diametrum maximum,
tetrahedrae, ornatae signo tri-radiato, eminenti, reticulatae, 5-7 reticulationibus
in facie externa, faciebus internis imperfecte atque irregulariter reticulatis, alatae,
alis ad 6.6μ latis, crenatis, verruculosis.

Monoecious, bright green, usually overlapping, often forming rosettes 5 mm.
across ; *thallus* once or twice furcate, 3-6 mm. long and 2-3 mm. broad, sulcate,
sulcus deeper anteriorly ; *scales* hyaline, incospicuous ; *rhizoids* simple and tuber-
culate, tubercles very prominent ; *cross-section* 10-15 times broader than high,
wings greatly attenuated on both the sides ; *air-spaces Ricciella* type ; *epidermal
cells* 1-layered, hyaline, spherical ; antheridial ostioles about 50μ above the thallus
surface ; *sporophytes* uniseriate, bulging conspicuously on the ventral surface ;
spore light brown, 65-85μ, tetrahedral with tri-radiate mark prominent, reticulate
*with 5-7 reticulations across the outer face, inner faces incompletely and irregularly
reticulate,* winged, wing upto 6.6μ wide, crenate, surface warty (Text-Fig. 1).

Coll : S. K. Pandé   *Loc.* Kamakhya Hills, Gauhati. Growing on laterite soil in intimate association with *R. billardieri.   Date* : 30.8.1952. PANDÉ COLLECTION No. 4798/4980 (Type).   Lucknow University.
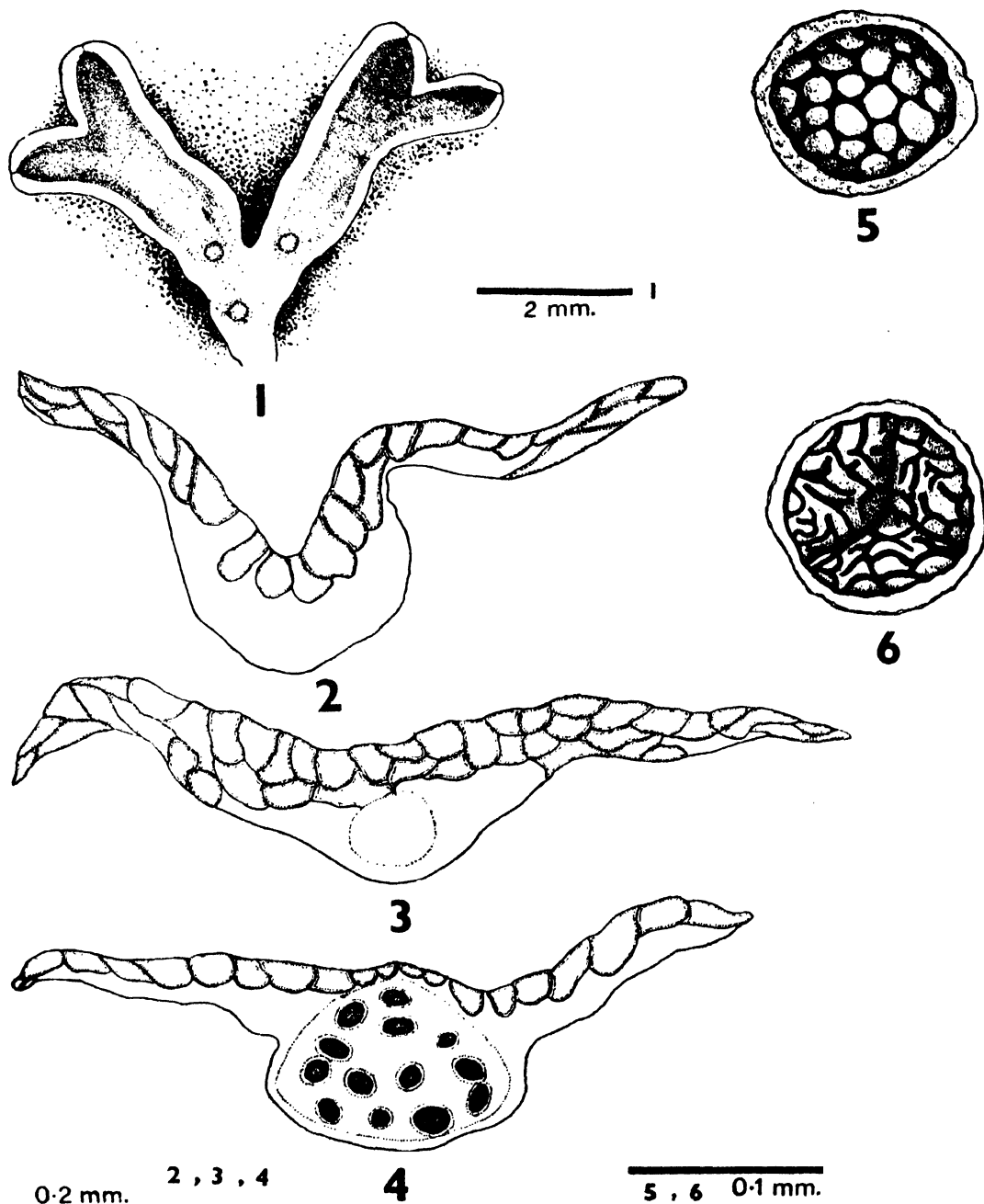
TEXT–FIG. 1.

Fig. 1.   Thallus, dorsal.   Fig. 2.   Cross-section of a thallus at apex.
Fig. 3.   Same, in the middle.
Fig. 4.   Same, at the base.   Note the attenuated wings (Figs. 2–4).
Fig. 5.   Spore, outer face.   Fig. 6.   Spore, inner faces.

The distribution of *Riccia attenuata* is rather interesting. This species has also been identified by the authors from a collection from Kandy, Ceylon, Pandé Collection No. 4689. Curiously enough the specimens from Ceylon are also associated with *R. billardieri* and the two species grow in intimate association on laterite soil.

IDENTIFICATION

In his monographic account of the genus *Riccia* Stephani (1900) recognized 46 species under subgenus *Ricciella* and divided them under the following groups:

"IX.   Frons tenerrima, membranacea.

X.   Frons magis costata, alis attenuatis costum late superantibus.

XI.   Frons angusta, magis incrassata, antice plana.

XII.   Frons magis incrassata, antice sulcata vel canaliculata.

XIII.   Frons valde incrassata, pro more duplo latior quam alta."

Unfortunately several of the species treated by Stephani (1900) have been shown in recent years to be unauthentic and great doubt has been expressed on their delimitation, sexuality and stability of identification. Quite a few were also incompletely described by him. However, in view of the compact nature of the treatment of the genus in Stephani's publication, it often proves helpful for purposes of identification.

The species under consideration belongs to section IX above being tender and membranous and also resembles section X in having greatly attenuated wings of the thallus.

In section IX Stephani (1900) treated the following 6 species : *R. membranacea* Gottsche et Lindenb., *R. welwitschii* St., *R. paraguayensis* Spruce, *R. ochrospora* M. et N., *R. amazonica* Spruce and *R. spruceana* St. Of these the three marked with an asterisk have been described by Stephani as *dioecious* and the other three as *monoecious*. Recently, however, Jones (1957) has reduced *R. welwitschii* as a synonym of *R. membranacea* and has described the latter to be monoecious. Thus four species need consideration in the present context, viz., *R. membranacea*, *R. paraguayensis*, *R. ochrospora* and *R. spruceana*. Of these *R. membranacea* is characterised by spore with truncate spines on the outer face and needs no attention and the remaining three differ in several important features (see Table I) from the species under consideration.

In section X also Stephani treated 6 species viz., *R. muscicola* St., *R. purpurascens* L. et. L., *R. crassifrons* Spruce, *R. donnellii* Aust., *R. subtilis* St. and *R. abnormis* St. All these species, except the last one, are *dioecious* and need no attention in this connection. It may also be pointed out here that recently the last species has been reduced by Jones (1957) to a synonym of *R. moenkemeyeri*, a species treated by Stephani in section XII. This species, too, differs in several important characters from the species in question (see Table I).

The species under consideration is also different from all the dioecious species of section IX and X of Stephani (1900) and even though some of the species may have been wrongly described by him with respect to their sexuality, other features differ significantly (Table II).

Some more species, belonging to the *Ricciella* section, not treated by Stephani are *R. cruciata* Kashyap (Kashyap, 1916, 1929), *R. cupulifera* Duthie (Duthie and Garside, 1936), *R. compacta* Garside (Duthie and Garside, 1939), *R. nipponica* Hatt. (Shimizu and Hattori, 1953), *R. perssonii* Khan (Khan, 1955) *R. arnellii* Khan (Khan, 1957) and *R. volkii* Arnell (Arnell, 1957). All these also differ in the vegetative and spore character from the species under consideration (see Table I).

TABLE I

No.	Name of Species	Thallus size	Colour	SPORE			
				Size	Number of reticulations	Size of reticulations	Wing
1.	R. *membranacea*	Upto 6 mm. long and 1–4 mm. broad	Light brown	30–50μ	Absent. Numerous slender truncate spines.	—	Absent
2.	R. *ochrospora*	4 mm. long, furcate	×	×	×	×	×
3.	R. *paraguayensis*	10 mm. long, trifurcate.	×	60μ	?	5μ	Wide, crenulate
4.	R. *spruceana*	ca. 10 mm. long irregularly furcate	Reddish-brown	51μ	×	5μ	Erose-dentate
5.	R. *moenkemeyeri*	Upto 10 mm. long	Yellow-brown	60–85μ often 100μ	9–15	10–12μ	Entire, thickened at the margin often with pits at one or two angles.
6.	R. *cruciata*	Upto 5 mm. long	Brown	60μ	3–4	20μ	Crenulata
7.	R. *cupulifera*	Upto 8 mm. long	Brown	70μ	5–8	10μ	Crenate
8.	R. *compacta*	Upto 10 mm. long	Dark brown	80μ	6–8	12μ	,,
9.	R. *nipponica*	Upto 5 mm. long	Brown	60–80μ	4	15–20μ	Finely dentate
10.	R. *volkii*	Upto 7 mm. long and 1 mm. broad	×	×	×	×	×
11.	R. *attenuata*	Upto 6 mm. long and 3 mm. broad	Deep brown	65–85μ	5–7	9–13μ	Crenate

×   Characters not described.

6

TABLE II

No.	Name of Species	Thallus size	SPORE					
			Colour	Size	Number of reticulations	Size of reticulations	Wing
1.	*R. amazonica*	Upto 6 mm. long	×	34μ	×	×	×
2.	*R. muscicola*	8 mm. long	×	60μ	×	×	×
3.	*R. purpurascens*	×	×	×μ	×	×	×
4.	*R. crassifrons*	Upto 12 mm. long	Brown	51μ	×	12μ	×
5.	*R. donnellii*	Upto 25 mm. long	Black	136μ	×	×	×
6.	*R. subtilis*	Upto 12 mm. long	×	×	×	×	×
7.	*R. attenuata*	Upto 6 mm. long and 3 mm. broad	Deep	65–85μ	5–7	9–13μ	Crenate

× Characters not described

The specimen of *Riccia* from Kamakhya Hills, Assam, has, therefore, been referred to a new species, *Riccia attenuata* Pandé *sp. nov.*

2.   *Riccia beyrichiana* Hampe

R. beyrichiana grows widely in North America (Haynes, 1920 ; Schuster, 1953) and also in Europe (Macvicar, 1926 ; Müller, 1905-11 ; Van den Berghen, 1955 etc.). This plant has not earlier been reported from the subcontinent of Asia. The species, however, is apparently very common in Assam and its discovery from this area raises the possibility of its being wider in distribution than recognized at present. It has been recorded here for the first time from India. The illustrated account presented below gives the salient features, based on the study of the Indian specimens :

Monoecious, glaucous green, usually forming over-lapping patches, occasionally developing incomplete rosettes ; *thallus* upto 4 mm. long and 2 mm. broad, 2-3 times dichotomously branched, sulcate, sulcus deep and acute anteriorly, broad and shallow behind, almost disappearing posteriorly, margin raised and convex, naked, *scales* hyaline, often violet ; *cross-section* 2-3.5 times as broad as high, wing gradually ascending ending in recurved margin ; *epidermal cells* pyriform-papillate, thin walled, 1-layered ; antheridial ostiole about 80μ above surface, hyaline ; *sporophytes* uniseriate, prominent dorsally ; *spore* dark brown, 90-130μ in diameter, reticulate with 7-10 reticulations across the outer face, reticulations upto 15μ wide, papillate in profile, winged, wing crenate, upto 6μ wide (Text-Fig. 2).

Coll : S. K. Pandé. *Loc :* Compound of the bungalow of Mr. Mustafi growing on moist soil. *Date :* 1.9.1952. PANDÉ COLLECTION No. 4696, 4697, 4699, Lucknow University.

The size of the thallus in *R. beyrichiana* from Assam is extremely variable. Whereas PANDÉ COLLECTION No. 4696 shows incomplete rosettes with segments upto 5 mm. in length, Nos. 4697 and 4699 show segments hardly exceeding 2 mm. and both are mature with fully developed sporophytes.

R. beyrichiana is known to occur both in ciliate and non-ciliate forms in Europe (Macvicar, 1926 ; Müller, 1905-11 ; Van den Berghen, 1955). Our specimens are non-ciliate. Possibly only the non-ciliate form grows in Assam or due to moist conditions prevailing during the time of collection cilia were not present.

3.   *Riccia discolor* L. *et* L.

R. discolor is apparently rare.

Coll : S. K. Pandé. *Loc :* Kamakhya Hills, Gauhati. On laterite soil. *Date :* 30.8.1952. PANDÉ COLLECTION No. 5000. Lucknow University.

4.   *Riccia billardieri* Mont. *et* N.

R. billardieri is comparatively more common than *R. discolor*. It occurs growing intermingled with *R. attenuata* Pandé. Often, however, pure growth of this species is also noticed.

.   *Coll :* S. K. Pandé. *Loc :* Kamakhya Hills, Gauhati. Growing on laterite soil in intimate association with *R. attenuata*. PANDÉ COLLECTION No. 4978, 4980. Lucknow University. *Loc :* Compound of the Cotton College, Gauhati. *Date :* 1.10.1952. No. 4979. PANDÉ COLLECTION, Lucknow University. (Pure growth).

5.   *Riccia gangetica* Ahmad

R. gangetica is not so common in Assam as *R. discolor* and *R. billardieri*.

Coll : S. K. Pandé. *Loc :* In the compound of the bungalow of Mr. Mustafi. *Date :* 1.10.1952. No. 4990. PANDÉ COLLECTION. Lucknow University.

2 mm.   '7 , 8   ———— 9 , 10   ———— 11
0·5 mm.   0·1 mm.
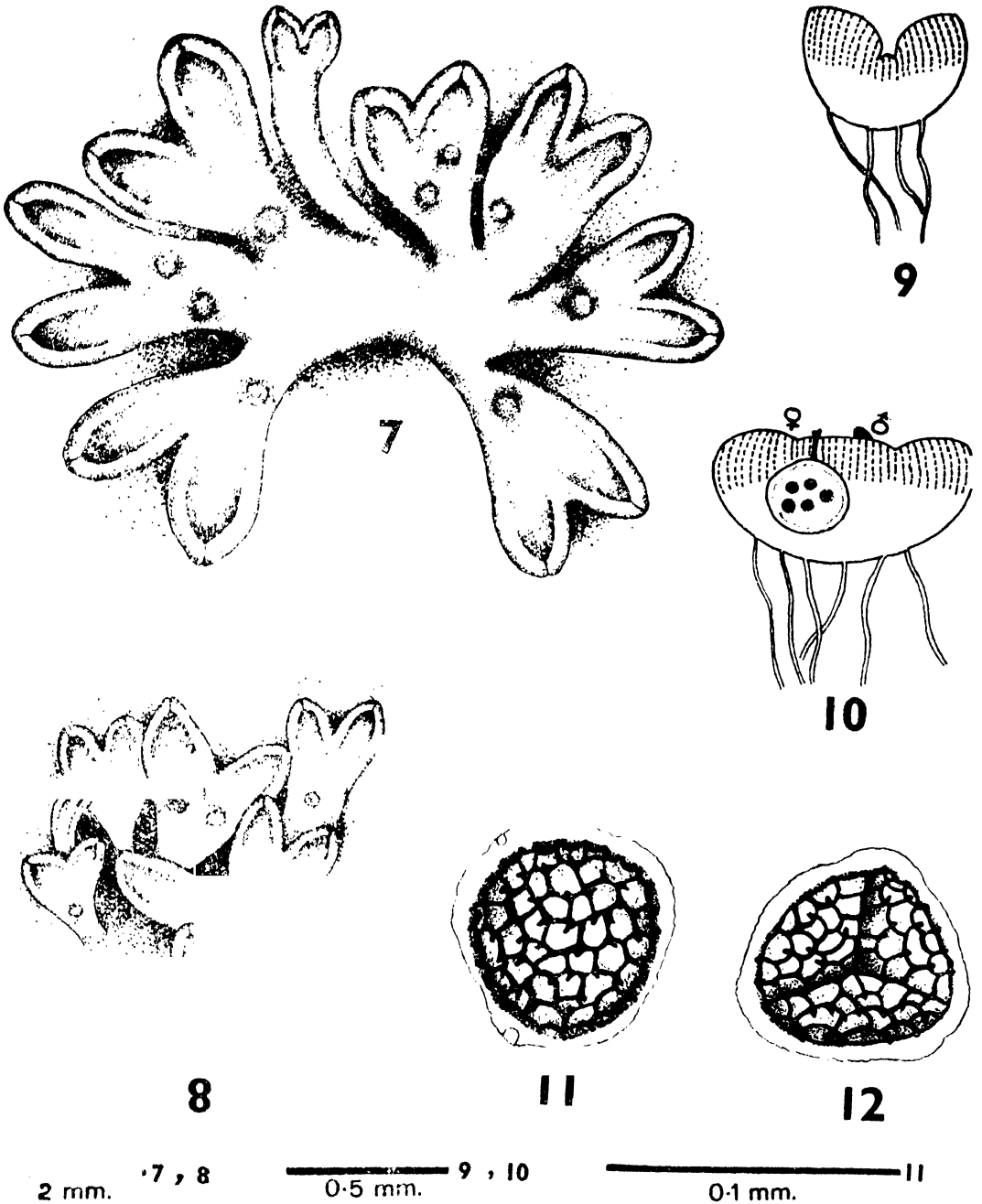
TEXT-FIG. 2.

Fig. 7.   Habit, an incomplete rosette.
Fig. 8.   Habit, overlapping thalli.
Fig. 9.   Cross-section of a thallus at apex.
Fig. 10. Same, in the middle.
Fig. 11. Spore, outer face.
Fig. 12. Spore, inner faces.

6.   *Riccia sorocarpa* Bischoff.

R. sorocarpa has already been reported as new to Indian flora by Udar (1956) on the basis of the collection made by Pandé from the Sikkim Himalaya in 1941. Salient features along with illustrations have also been given.

Coll : S. K. Pandé.   *Loc :* In the compound of the dak bungalow in Phaloot (12-13,000 ft.). *Date :* 28.9.1941. PANDÉ COLLECTION No. 2436. Lucknow University.

7.   *Riccia huebeneriana* Lindenb.

Syn : *R. kashyapii* Kachroo in *Sci. & Culture,* **20**, 98-101.

R. huebeneriana was also reported as new to Indian flora by Udar (1956) along with *R. sorocarpa* from the same collection and salient features and illustrations were also given.

R. kashyapii Kachroo (Kachroo, 1954) does not appear to differ significantly from *R. huebeneriana* Lindenb. either in vegetative structure or features of spores and has consequently been reduced to a synonym of the latter. Unfortunately it has not been possible for us to secure the type specimens of *R. kashyapii* but the excellent account and illustrations of this species given by Kachroo (1954) leaves absolutely no doubt about its identity with *R. huebeneriana* which occurs in Assam (see Udar, 1956).

Coll : S. K. Pandé.   *Loc :* About 7 miles from Darjeeling on moist ground (2,500 ft.) on a foot path leading to Badampton. *Date :* 27.9.1941. PANDÉ COLLECTION No. 2446. Lucknow University.

REFERENCES

Arnell, S. (1957).   Hepaticae collected in South West Africa by Prof. Dr. O. H. Volk.   *Mitteilung. der Bot.* Staatss., **16**, 262-72.

Chopra, R. S. (1938).   Notes on Indian Hepatics. II.   Sikkim Himalaya and Bengal. *Proc. Indian Acad. Sci.*, **8**, B, 427-39.

Duthie, A. V. and Garside, S. (1936).   Studies in South African Ricciaceae.   *Trans. Roy. Soc. S. Afr.*, **24**, 93-133.

———— (1939).   Studies in South African Ricciaceae.   *Trans. roy. Soc. S. Afr.*, **28**, 17-28.

Griffith, W. (1849).   *Notulae ad Plantae Asiaticae*, Calcutta.

———— (1849a).   *Icones Plantarum Asiaticarum*, Part II, Calcutta.

Haynes, C. C. (1920).   Illustrations of six species of *Riccia*, with the original descriptions. *Bull. Torr. bot. club.*, **47**, 279-87.

Jones, E.W. (1957).   African Hepatics. XIII.   The Ricciaceae in Tropical Africa.   *Trans. Brit. bryol. Soc.*, **3** : 208-227,

Kachroo, P. (1950).   Studies in Assam Hepaticae. I.   *J. Gauhati University,* **36**, 18-20.

———— (1952).   Distribution of liverworts in Assam.   *Sci. and Cult.*, **18**, 284-85.

———— (1954).   Studies in Assam Hepaticae. III.   On a new species of *Anthoceros* L. and *Riccia* L., *Sci. and Cult.*, **20**, 98-101.

Kashyap, S. R. (1916).   Liverworts of the Western Himalayas and the Punjab.   *J. Bombay nat. Hist. Soc.*, **24**, 343-50.

———— (1929).   *Liverworts of the Western Himalayas and the Punjab plains,* **1**, Lahore.

Khan, S. A. (1955).   *Riccia perssonii* S. A. Khan : A new interesting species from East Pakistan. *Svensk bot. Tidskr.*, **49**, 432-36.

———— (1957).   Studies in Ricciaceae of East Pakistan. I.   New and little known species of *Riccia*. *Bryologist*, **60**, 28-32.

Macvicar, S. M. (1926). *The Students' Hand book of British Hepatics*, London.

Mitten, W. (1861). Hepaticae Indiae Orientalis : An enumeration of the Hepaticae of East Indies. *J. Proc. Linn. Soc.*, London, **5**, 89–109.

Müller, K. (1905-11). Die Lebermoose Deutschlands, Oesterreichs u.d. Schweiz. *Rabenhorst's Kryptogamenflora.* Zweite Auflage. Bd. 6, Jena.

Pandé, S. K. (1958). Some aspects of Indian Hepaticology. Presidential Address to the Annual Meeting of the Indian Botanical Society. *J. Indian Bot. Soc.*, **37** : 1–26.

Pandé, S. K. and Udar, R. (1957). Genus *Riccia* in India—I. A re-investigation of the taxonomic status of the Indian species of *Riccia*. *J. Indian bot. Soc.*, **36**, 564–79.

Shimizu, D. and Hattori, S. (1953). Marchantiales of Japan. I. *The J. Hattori bot. lab.*, No. **9**, 32–44.

Schuster, R. M. (1953). Boreal Hepaticae : A manual of the Liverworts of Minnesota and adjacent regions. *The American Midland Naturalist*, **49**, 257–684.

Stephani, F. (1900). *Species Hepaticarum*, 1, Genève.

———— (1917–24). *Ibid.* **6**, Genève.

Udar, R. (1956). On two species of *Riccia* new to Indian flora. *Curr. Sci.*, **25**, 232–33.

———— (1957). On the synonymy of some Indian species of *Riccia*. *Curr. Sci.*, **26**, 20–22.

Van den Berghen, C. (1955). *Flore Générale de Belgique. 1. Jardin bot. de l'état*, 1–110.

FUNCTIONAL MORPHOLOGY OF THE CLOACA OF *VARANUS MONITOR* (LINNAEUS) IN RELATION TO WATER ECONOMY

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ABSTRACT

   Morphology of the cloaca of *Varanus monitor* has been described. There is a structural difference in the cloaca of male and female *Varanus* and consequently the mechanism of the flow of liquid urine to the place of dehydration is different in the two sexes. Uric acid is the main urinary component present to the extent of 88 per cent. There is a corresponding decrease in the percentage of allantoin and creatine.

INTRODUCTION

   One of the most important problems of animal life is to maintain inside the body just the right amount of water. For animals that have direct access to water and can get water in plenty it is easy to maintain the constancy of water balance in the body. But water conservation is a real problem in animals that live in desert conditions where water is scarce. On the basis of water conservation Buxton (1933) categorised terrestrial insects into 'spenders' and 'savers'. This categorisation can be extended to the terrestrial animals in general. 'Spenders' live in environments where water is easily available, they are able to make up losses by the oral intake and are characterised by a high water turn over and there is little provision for conservation. The example which can be quoted for this category is the common pond-turtle, *Lissemys punctata* studied by the author in relation to urinary excretion (Seshadri, 1956*b*). The 'savers' live in dry environments, take in almost dry food, have a relatively low body-water content and are resistant to starvation. The common house lizard, *Hemidactylus flaviviridis* (Seshadri, 1956*a*) and the spiny-tailed lizard, *Uromastix hardwickii* (Seshadri, 1957*b, in press*), belong to the second category. Highly concentrated urine is an important feature for such water economy and Nature has developed a unique mechanism for the conservation of water in lizards, in which urine gets dehydrated to such an extent that solid urine is evacuated. This is to ensure that no loss of water occurs by such excretion.

   Functional morphology of the cloaca of *Varanus monitor* is presented in this paper where morphology of the cloaca, the mechanism of the flow of liquid urine to the place of dehydration and the chemical composition of urine are dealt with. The methods for collecting the urinary pellets and the technique adopted for the study of morphology of cloaca and the chemical analysis of urine are similar to those described previously by the author (Seshadri, 1956*a, b*).

MORPHOLOGY OF THE CLOACA

   In lizards the kidneys of the two sides are generally united at the caudal end and form a V-shaped structure, but in *Varanus monitor* they do not meet and remain completely separate. They do not extend so far behind the tail region as in other lizards. The kidneys are fan-shaped and lobulated and resemble to some extent the kidneys of pond-turtle, *Lissemys punctata*, each lobe in the kidney having a number of finger-like projections. The ureters from the kidney open

into the urodaeum.   In male, the ureters and the *vasa deferentia* of each side join and open on a prominent urinogenital papilla, while in female the ureters open on separate urinary papillae.   There is no urinary bladder in *Varanus*.

As in other lizards, the cloaca is three-chambered and is of the typical saurian type.   The disposition of the cloacal chambers is slightly different in the case of male and female.   In male the urodaeum as usual is in a line between the corprodaeum in front and proctodaeum behind, while in female the urodaeal chamber is slightly displaced and is directly above or dorsal to the coprodaeum (Fig. 1).

The coprodaeum is demarcated from the rectum by a large sphincter and the lining in this region is closely set with longitudinal folds.   The ventral wall of the coprodaeum has a few distantly arranged longitudinal folds.   In between the longitudinal folds are a series of small, transverse folds, arranged in a definite pattern.
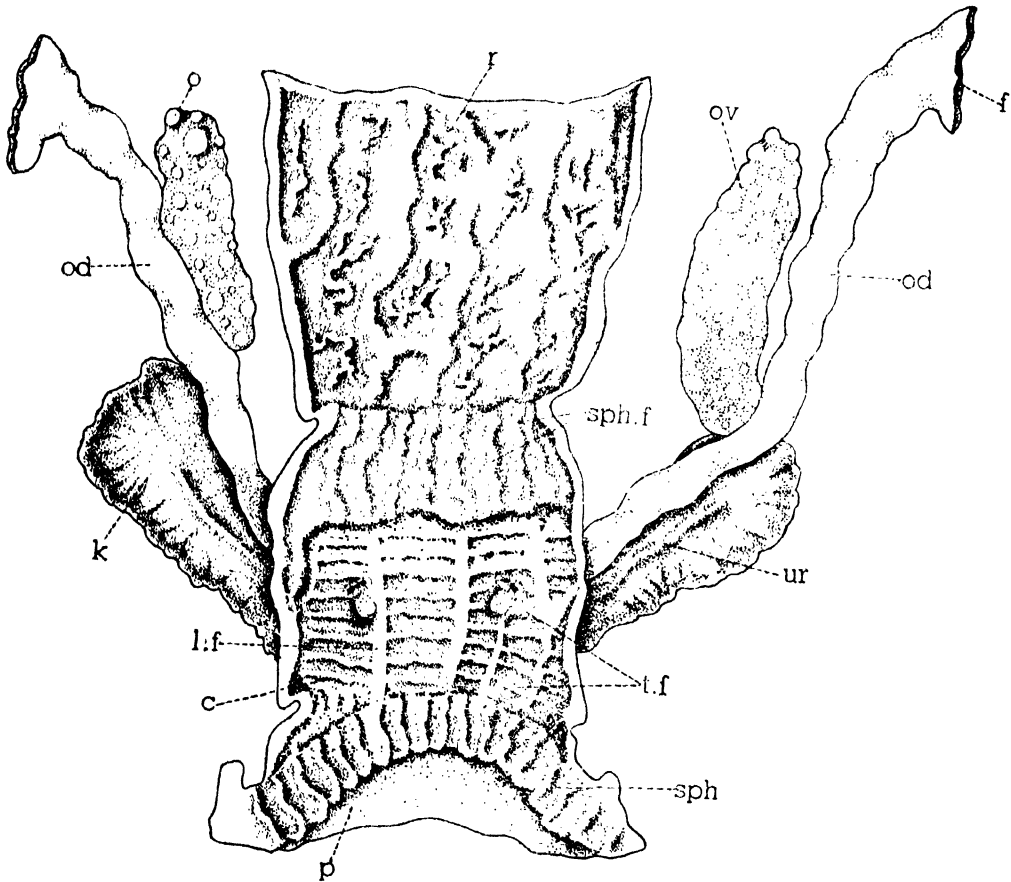
TEXT-FIG. 1.

Varanus monitor.   Dissection of the female urinogenital system.
c., coprodaeum ; *f.*, funnel ; *k.*, kidney ; *l.f.*, longitudinal fold ; *o.*, ova ; *od.*, oviduct ; *ov.*, ovary ; *p.*, proctodaeum ; *r.*, rectum ; *sph.*, sphincter between coprodaeum and proctodaeum (copro-proctodaeal sphincter) ; *sph.f.*, folds in the region of sphincter ; *t.f.*, transverse folds of coprodaeum ; *ur.*, ureter.

In male, the urodaeum is in the same line as the coprodaeum.   The ureters and *vasa deferentia* open on urinogenital papillae arising from the dorso-lateral wall of the urodaeum.   These papillae are so raised as to be in level with the

fold which demarcates the urodaeum and coprodaeum. In the region of sphincter are present a number of closely set folds and between them the longitudinally disposed grooves.

In female the urodaeal chamber instead of being caudad to the coprodaeal chamber appears as a diverticulum of the dorsal wall of the cloaca which comes to lie just dorsal to the coprodaeum. When cloaca is opened on ventral side the urodaeum remains completely hidden under the coprodaeum, and thus the coprodaeum is in direct communication with the proctodaeum. The transverse fold and the sphincter between the coprodaeum and urodaeum in the male appear to separate the coprodaeum and proctodaeum in the female. So in male cloaca it can be named as a uro-coprodaeal sphincter and in the female the copro-procto-daeal sphincter. Longitudinal folds and grooves are placed in the region of copro-proctodaeal sphincter.

A longitudinal incision in the dorsal wall of the coprodaeum exposes the dorsally located urodaeal chamber. On the dorso-lateral sides are, in two pairs, the genital and the urinary openings, the urinary apertures are situated on papillae. The urinary aperture of each side is placed a little below the opening of the oviduct of the same side. Dorsal wall of urodaeum is slightly raised creating corresponding depressions in the dorso-lateral walls. As already stated, the urinary and the genital apertures are present on the dorso-lateral sides and are located in the respective dorso-lateral concavities. The median raised portion of the dorsal wall has a few transversely placed folds.

On the dorso-lateral walls of urodaeum, in the female, are a few longitudinal folds which run along a definite course. They emerge from below the opening and then pass on to the ventral wall of the urodaeum. The dorso-lateral folds are directed towards the coprodaeum or cephalad and finally merge into the folds in the region of the copro-proctodaeal sphincter. These folds and grooves between them are the uro-coprodaeal folds and grooves (Fig. 2).

The median raised portion of the dorsal wall of urodaeum continues back and finally merges with the dorsal wall of the proctodaeal chamber. The proctodaeum also has only a few mild transverse folds and the wall presents a pitted appearance.

MECHANISM OF THE FLOW OF URINE

Due to the structural difference of cloaca in the male and female, it necessarily follows that mechanism of the flow of liquid urine also differs in the two sexes. In male the mechanism of the flow of urine from the urodaeum to the coprodaeum, where urine gets dehydrated, is much simpler than in the female. In the absence of a urinary bladder the urine passes directly into the coprodaeum and in this respect the mechanism is almost the same as in *Hemidactylus* where there is a direct flow of urine from urodaeum into the coprodaeum. The urino-genital papillae are also directed slightly forward and thus facilitate flow of urine into the coprodaeum, along the grooves present between the folds in the region of the uro-coprodaeal sphincter.

The flow of liquid urine in the female, from the urodaeal chamber to the ventrally located coprodaeum, is facilitated by the well-developed uro-coprodaeal folds and grooves. The natural tendency of liquid is to flow towards the posterior chamber, especially when the anterior chamber is not placed in line with it. The dehydration of urine takes place in the coprodaeal chamber, so the liquid is directed to this chamber. The ureters bring urine from the kidneys to the urodaeum. As liquid urine trickles down from the urinary papilla it is taken up by the uro-coprodaeal grooves which emerge from the region of the papilla in the dorso-lateral concavity. The grooves and folds arising from the base of the papilla are all directed towards the coprodaeum and this arrangement facilitates the flow of

liquid urine into the coprodaeum. The presence of folds modified to direct the flow of urine towards the chamber of dehydration is somewhat like that seen in *Uromastix*, in which case the presence of a bladder makes the arrangement slightly different. The folds and grooves between them form an inlet and outlet for the liquid to flow into the bladder and out of it towards the coprodaeum. But in *Varanus*, owing to the total absence of a urinary bladder there is a direct flow of urine into the coprodaeum.
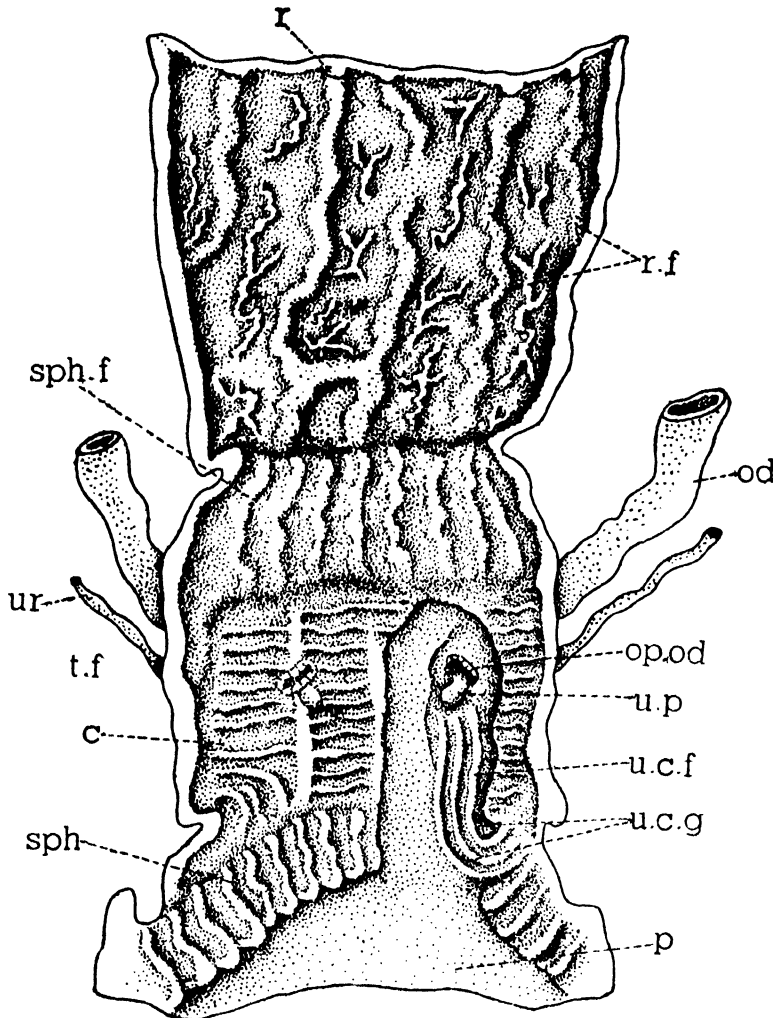
TEXT-FIG. 2.

Varanus monitor. Diagrammatic sketch to illustrate the position of urodaeum and uro-coprodaeal folds leading from urinary papilla to coprodaeum.
c., coprodaeum ; *od.*, oviduct ; *op. od.*, opening of oviduct ; *p.*, proctodaeum; *r.*, rectum; *r.f.*, folds of rectum ; *sph.*, sphincter ; *sph.f.*, folds in the region of sphincter ; *t.f.*, transverse folds ; *u.c.f.*, uro-coprodaeal folds ; *u.c.g.*, uro-coprodaeal grooves ; *u.p.*, urinary papilla ; *ur.* ureter.

Another possible way by which liquid urine reaches the coprodaeum can be conjectured in the following way : in natural course the cloacal opening to the exterior remains tightly closed. As already stated, the liquid urine trickling from

the urinary papillae has a natural tendency to flow into the chamber, behind it, i.e. the proctodaeum. When sufficient liquid accumulates, the coprodaeal chamber dilates, and with its dilatation the transverse fold in the region of the uro-coprodaeal sphincter, which is more prominent on the dorsal side, presses towards the urodaeum, which closes the passage to the urodaeal chamber. The cloacal opening is closed and also the passage to the urodaeum is blocked so that the liquid urine has only one way to follow and that is to the coprodaeum. This is presumably achieved by the contraction of proctodaeum. An area of low pressure is already created by the dilatation which would facilitate the flow of liquid in that direction. By the contraction of proctodaeum, liquid is forced along the grooves in the region of the copro-protodaeal sphincter (female) into the coprodaeum where it undergoes dehydration. The contraction of proctodaeum, on the basis of Alvarez's theory, starts an antiperistaltic movement of the type noticed in the case of *Hemidactylus*. Muscles of the posterior chamber develop a higher metabolic potential due to the presence of urine ; thereby the wave of contraction moves from the posterior to the anterior chamber.

The coprodaeal chamber is fairly large and can hold urine and retain it for a longer period. Transverse folds in the coprodaeum increase the surface area for absorption of water. A large chamber, lined by a number of folds, would prove an efficient device for the extraction and absorption of a maximum amount of water from the urine to convert it into a solid pellet and thus conserve the available quantity of water.

CHEMICAL COMPOSITION OF URINE

Dehydrated urinary pellet in a normal sized *Varanus* is like a small piece of lime stone. Some of the freshly excreted pellets are slightly yellowish in colour while most of them are pure white. The yellowish colour is due to a type of urinary pigment, very much like urobilin.

Chemical composition of a pellet of urine of *Varanus monitor*:

Constituents	Percentage per gm. of urine
Uric acid	88%
Creatine	2.2%
Creatinine	traces
Ash	5.0%
Moisture	2.0%
Allantoin	3.0%

The ash contains traces of sodium, chloride, carbonate, present in the form of sodium chloride, sodium carbonate, and calcium.

Uric acid is the main urinary component. The presence of uric acid to the extent of 90 per cent in *Varanus* is the highest percentage observed by the author in some of the lizards examined. Khalil (1951) noted in *Scinus officinalis* and *Chalicides ocellatus* 92 and 93 per cent of uric acid respectively, and correspondingly very small amount of creatine and allantoin. The result of chemical analysis of the urine of *Varanus monitor* tallies, to a great extent, with the above mentioned lizards. The point that the percentage of allantoin and creatine is small when the amount of uric acid is high, goes to prove that in reptiles uric acid is not only an end product of purine metabolism but also of protein. This is so because allantoin and creatine are protein metabolic products and they become correspondingly less.

ACKNOWLEDGEMENTS

REFERENCES

Buxton, P. A. (1923).   Animal life in Deserts.   London, Arnold Company.
———————— (1932).   Terrestrial insects and humidity.   *Biol. Rev.*, **7**, 275–320.
———— ——— (1933).   Climate and population.   *Trans. roy. Soc. trop. Med.* and *Hyg.*, **26**, 325–364.
Khalil, F. (1951).   Excretion in reptiles. IV. Nitrogen constituents of the excreta of lizards. V. *Biol. Chem.*, 175.
Seshadri, (1956a).   Urinary excretion in the Indian House lizard. *Hemidactylus flaviviridis* (Ruppell).   *J. zool. Soc. India*, **8** (1), 63–78.
——— ——— ——— (1956b).   Urinogenital organs and urinary excretion in the pond turtle. *Lissemys punctata punctata* Bonnaterre.   *ibid.*, **8** (2), 197–210.
——— ——— (1957).   Water conservation in *Uromastix hardwickii* (Gray) and the presence of Mullerian ducts in the male.   *ibid.*, **9** (2).

ERRATA

In Proc. Part B. Vol. 25. No. 1. (papers by S. RANJAN *and* B. BUDHRAJA entitled :

(1) A study of Nitrogen Fixation in Detached Root Nodules of Sunn Hemp and

(2) Metabolic changes in Potato Tubers under Anaerobiosis)

Consider—

      (i)   Text-Fig. 1   on p. 45 *in place of*
           Text-Fig. 1   on p. 40 and *vice versa,*

     (ii)   Text-Fig. 2   on p. 46 *in place of*
           Text-Fig. 2   on p. 41 and *vice versa*

and (iii)   Text-Fig. 3   on p. 47 *in place of*
           Text-Fig. 3   on p. 42 and *vice versa.*

STUDIES ON THE AGE AND GROWTH OF *CIRRHINA MRIGALA* (HAM.) FROM THE RIVER GANGA*

by V. G. JHINGRAN,** *Central Inland Fisheries Research Sub-Station, Allahabad*

CONTENTS

ABSTRACT

The population sampled for the investigation reported here was from the River Ganga at Buxar (Bihar, India). During the investigation, which was carried out, from June, 1952 to July, 1956, 5,179 specimens of *Mrigal* were examined for length-frequency distribution study, 4,369 scales from 807 specimens for scale study, 5,129 specimens for weight-frequency distribution study, 4,930 specimens for length-weight relationship study, and finally, 1,102 observations were made for length-girth relationship study. Such observations as progressively larger number of annuli on scales from fish of increasing length and lessening distance between successive annuli with progress in age, have furnished preliminary evidence suggesting application of scale-method to *Mrigal*. A close correspondence of early growth picture elucidated by scale and length-frequency distribution methods has furnished strong evidence in that direction. Annuli are believed to be laid in spring or summer months (March–June) as borne out by ovservations on scale margins. Support to this has been sought from the intensity of feeding studies and the length-frequency distribution study, which latter has also enabled a picture to be drawn of month to month growth upto about the third year-group stage. Starvation is held out as a probable cause of annulus formation. Detailed growth for first three years, absolute growth picture in length for twelve years and relative growth for seven years of the life of *Mrigal* have been worked out. Weight-frequency distribution has been studied and absolute growth in fish weight upto twelve years of its life and relative growth for seven years have been found. Total length and weight relationship and total length and girth relationships have been statistically worked out. A future course of study and conservation of *Mrigal* fishery have been discussed.

INTRODUCTION

Studies leading to the elucidation of the age and growth of commerically important species of fish are of vital significance in evolving effective policies for the management and conservation of their fisheries. *Cirrhina mrigala* (Ham.) is well known to sustain not only a very important inland capture fishery of India

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but is also a fish of great cultural value. Neither has any reliable age indicator of this species hitherto been known nor has its growth in the rivers of the country been studied. Some information, however, on its growth rate in confined waters is available (Bukht, 1940 ; Basu, 1946 ; Chacko and Ganapati, 1951). This paper initially presents evidence on the validity of certain markings on the scales of *Mrigal* as indicators of its age (Jhingran, 1957). Growth of the species, by utilising the scales as well as by adopting Petersen's length-frequency distribution method, is then delineated. Growth in weight has also been investigated and size-weight and size-girth relationships have been worked out. Finally, conservation of the species is discussed. A number of historical reviews and bibliographies on age determination of fishes by means of scales, which, however, largely pertain to species of temperate regions, is available in the literature, notable among which are those by Baudelot (1873), Thompson (1904), Taylor (1916), Hutton (1921), Creaser (1926), Mohr (1927, 1930, 1934), Graham (1929) and Van Oosten (1929). The determination of the age of fish by means of scales has been so extensively used in temperate regions during the last four decades that a detailed description of the method seems unnecessary. Some of the well known commercially important fishes whose growth and life histories have been elucidated by scale studies are : salmon, trout, marine herring, halibut, plaice, flounder, sole, smelt, mackerel, sardine, eel, hake, haddock, cod etc. Considerable work has also been done on typical freshwater fishes notably in the United States of America and Canada. Numerous investigators like Hile (1931, 1936, 1941, 1942) : Foerster (1936) : Van Oosten (1923, 1929, 1937, 1938, 1939, 1941, 1942) : Beckman (1941, 1942, 1943a, b, 1949) : Ricker (1942) : Applegate (1943, 1947) : Jobes (1933, 1943, 1949a,b) : Carlander (1945a,b, 1948, 1950a,b) : Stroud (1948, 1949a,b) : Eschmeyer (1950) Sprugel (1953, 1955), Parsons (1953), Kennedy (1954a,b) : Alvord (1953) and Godrey (1955), among many others, have successfully utilised the scales for age and growth studies of freshwater fishes. The little work on age determination, which has hitherto been done on Indian species, marine or otherwise, has largely been confined to the Indian sardine, *Sardinella longiceps* (Hornell and Naidu, 1924 ; Devanesan, 1943 ; Nair, 1949 and Chidambaram, 1950) and the Indian shad, *Hilsa ilisha* (Hora and Nair, 1940 ; Chacko et al. 1948 ; Chacko and Krishnamurti, 1950 ; Chacko and Dixitullu, 1951 : Raj, 1951 and Jones and Menon, 1951). Other species investigated are *Therapon jarbua* (Rao, 1934), *Psettodes erumei* (Rao, 1935), and *Rastrelliger kanagurta* (Chidambaram and Krishnamurti, 1951). Chevey (1930, 1932) studied a number of species of Indochina, Cochin-China and Cambodia and Devasundaram (1952) of *Mugil cephalus*. Sheshappa and Bhimachar (1951, 1954) studied the scales of *Cynoglossus semifasciatus*, Pillay (1954) of *Mugil tade*, Pantulu (1956) of *Anguilla bengalensis* and Sarojini (1957) of *Mugil parsia*. Menon (1953) has reviewed the work done on age and growth of tropical and sub-tropical fishes. Possible annular significance of certain markings on the scales of fishes of Indian waters has been made by quite a number of workers, but the validity of such markings as true indicators of age has not been critically established except in very few cases.

MATERIAL AND METHODS

The population sampled for the investigation reported here was from the River Ganga at Buxar (Bihar, India), where investigations to elucidate the growth of *Cirrhina mrigala* (Ham.) were commenced in June 1952. As hitherto no method had been known to determine the age of *Mrigal*, the basic approach for the study of the growth of this species was by following Petersen's length-frequency distribution method inspite of its certain well known drawbacks, this being the only method possible under the circumstances prevalent then. However, early during the course of this work certain markings were observed on the scale surface, which

as subsequent work revealed, might be used to determine the age of this species. A total of 5,179 specimens of all available sizes in the commercial catches was measured during the period—June, 1952, to July, 1956. These monthly data were utilised for verifying the results obtained by scale studies of this species. For the latter, 4,369 scales from 807 specimens of the size range—103.00 mm. to 1,010.00 mm. in total length—were examined. All the scales were picked up for study from the region directly below the dorsal fin and above the lateral line. In a few cases the scales on the lateral line itself were also taken. Since the scales attain a fairly big size and are translucent, the only optical aid occasionally felt necessary to demarcate annuli like markings was an ordinary magnifying lens. The scales of juvenile fish and the detailed disposition of circuli in the annular zone in relation to those preceding or following such zones were also examined under the microscope. In a majority of the cases, however, the checks, especially the earlier ones, were clearly visible to the naked eye when the scales were held against an open window or a door in an otherwise darkened room. Measurements, along the median longitudinal axis of the linear distances between the approximate centres of the scale foci and the mid-points of the various annuli, were taken directly on the scale surface by means of a scale divided into half-milli-metres. Lengths attained by the fish at the time of formation of the various annuli were back calculated for each specimen individually using the formula : $L_1 = \dfrac{L \times l_1}{l}$

where L is the length of the fish from which scales were taken ; L_1 is the length of the fish at the time of annulus formation ; l is the length of the scale from the focus to the anterior apex of the scale and l_1 the distance between focus and each annulus. A justification for the use of this formula is discussed later in this paper. A frequency table, showing sizes attained by *Mrigal* at different ages of its life was prepared, graphically represented, and compared with the correspond-ing data and results obtained by the study of the length-frequency distributions. Finally, the growth picture of the species has been elucidated by pooling the available evidences together. The markings, which, in the present study, have been interpreted as annular in nature, have been referred to as "annuli" in this paper, although their being conclusively termed so is subject to "known age method" being applied (Jhingran, 1957).

The variables, length and weight, of fishes are closely related, and are described by a high positive coefficient of correlation (Jhingran, 1952). Attempts were, therefore, made to see whether corroboration of the scale method could be got from a study of the weight-frequency distribution, in a similar manner as that obtained from the length-frequency distribution. Weight data on 5,129 specimens of *Mrigal*, taken in different months of the year during the course of the study, were pooled for the corresponding months and graphically represented for study. Length-weight relationship was studied by the method of the least squares, and the fomula derived to convert length values into weight or *vice versa*, represents a rough and overall correlation between total length in millimetres and weight in grams of combined sexes varying in length from 78 mm. to 1016 mm. regard-less of the maturity of gonads or gut contents. The data on 4,930 weight records for fish of corresponding lengths, to the nearest gram, were pooled, and the average weight for fish at each millimetre interval was computed. A correlation table was drawn to determine the formula correlating the two variables. Total length and girth relationship was similarly studied with the aid of a correlation table by pool-ing the entire data, comprising 1,102 observations of the total length range— 200 mm. to 1,010 mm.—into classes of one millimetre interval and determining the average of the corresponding girth values. An approximate overall length-girth relationship was thus obtained. Determination of sex and stage of maturity, which obviously affects girth dimensions, was not considered essential, since practical

fishery considerations require the fixation of only a minimum size limit. Available facilities in the field also did not permit the determination of sex.

VALIDITY OF THE SCALE METHOD IN *Cirrhina mrigala* (Ham.)

Theoretically a fundamental tenet of the application of the scale method to the study of growth by back calculation is the occurrence of isometric growth between scale and body lengths. This has usually been assumed to be so, though wherever the relationship has been investigated it has been found to be more or less curvilinear, and some sort of a correction is, strictly speaking, needed to arrive at accurate back-calculated figures. Such a relationship is also to be expected because scales from subsequent to the hatching of the fish, when the latter has already attained a certain length and the later growth may not be mathematically isometric. Figure 1 presents a scatter diagram, on an arithmetic plot of 764 observations on total length of *Mrigal* of the size range—180 mm. to 1,010 mm.— plotted against corresponding scale lengths, showing a length range of 4 mm. to 23.5 mm., measured from the focus to the posterior extremity of the scale in the median antero-posterior axis. The scatter of points does not indicate a rectilinear relationship. Perhaps two or more straight lines (Smith, 1955) or a curve fitted to the points, would better describe the scale-body relationship, but, in view of only a slight smooth curvature of the trend of the scatter in the case of *Mrigal*, and absence of a clear indication of a flexion point in the available data, neither of the two procedures has been adopted. The gentle curvature in the scatter diagram has been preferred to be neglected and a rectilinear form of relationship between scale and body lengths has thus been virtually assumed in the present study. A direct proportion has been accepted to facilitate handling a large volume of data without sacrificing scientific accuracy from the practical fisheries viewpoint. This procedure is further justified by a high coefficient of correlation, (+0.96) between scale lengths (focus to margin) and fish lengths

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Plate V :

Scale 1 : Total length of fish :
368.3 mm.
Date of collection :
3-7-1952
Shows one check.

Scale 2 : Total length of fish :
596.9 mm.
Date of collection :
27-12-1952
Shows two checks.

Scale 3 : Total length of fish :
736.6 mm.
Date of collection :
24-7-1952
Shows three checks.

Scale 4 : Total length of fish :
762.0 mm.
Date of collection :
29-12-1952
Shows four checks.

Scale 5 : Total length of fish :
914.5 mm.
Date of collection :
9-7-1952
Shows five checks.

Scale 6: Total length of fish :
978.0 mm.
Date of collection :
3-7-1952
Shows six checks.

Scale 7 : Total length of fish :
914.0 mm.
Date of collection :
28-6-1952
Shows seven checks.

(Two lines, one above the other, at the extreme left lower margin of each print shows the total length of the actual corresponding scale at its antero-posterior axis in reation with the photograph arranged along with the same axis.)

1

2

3

4

5

6

7
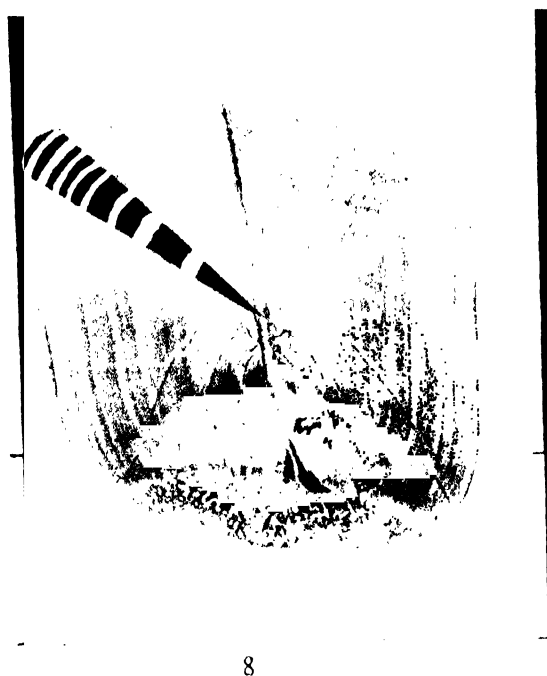
8

9

Plate VI   Scale 8 : Total length of fish :
946.5 mm.
Date of collection :
20-11-1952
Shows eight checks.

Scale 9 : Total length of fish :
997.3 mm.
Date of collection :
14-11-1952
Shows nine checks.

(Two broken lines one above the other, at the extreme left lower margin of each print, show the total length of the actual corresponding scale at its antero-posterior axis in relation with the photograph arranged along the same axis.)

10

11

Plate VII. Scale 10 : Total length of fish :   Scale 11 : Total length of fish :
984.6 mm.   1010.0 mm.
Date of collection .   Date of collection :
17-3-1954   18-11-1953
Shows ten checks.   Shows about 13 checks.

(Two broken lines one above the other, at the extreme left lower margin of each print, show the total length of the actual corresponding scale at its antero-posterior axis in relation with the photograph arranged along the same axis.)

12 13

15

14
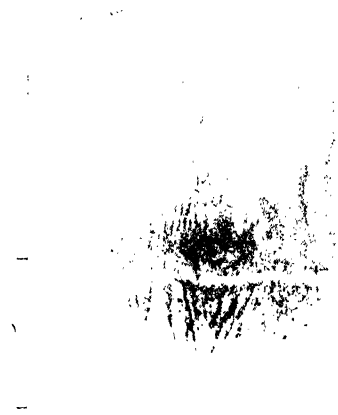
16

(total length) found in *Mrigal*. The value of *r* would undoubtedly be still closer to unity were scales from exactly the same location taken for the study of this
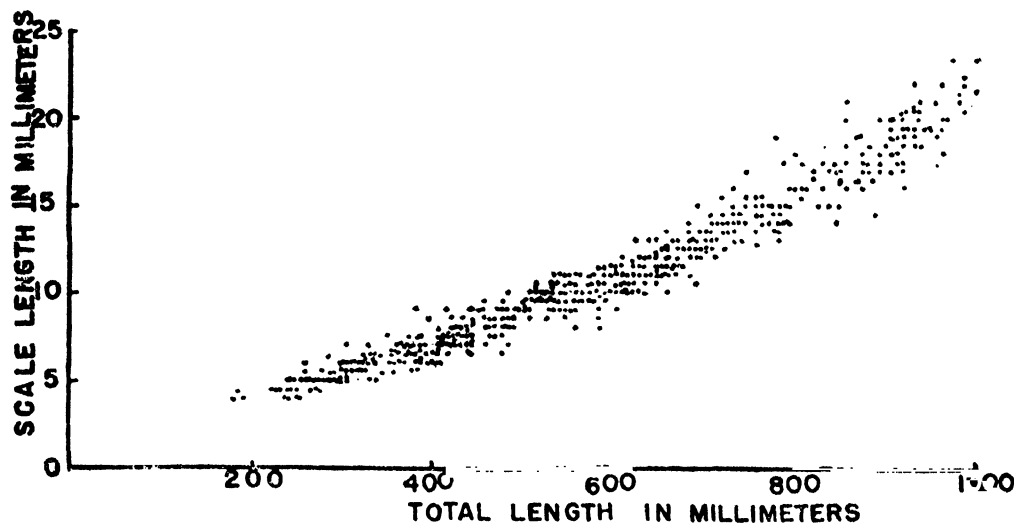
TEXT-FIG. 1.

Scatter diagram showing a plot of 764 observations on total length of *Mrigal* varying in size from 180 mm. to 1010 mm. against corresponding scale lengths (focus to anterior margin) varying in size from 4 mm. to 23.5 mm.

Plate VIII :

Scale 12 : Total length of fish :
971.9 mm.
Date of collection :
21-12-1952
Close view of a sector of the scale showing breaks in the continuity of circuli in the annular zone.

Scale 13 : Total length of fish :
762.0 mm.
Date of collection :
5-4-1955
Shows marginal annulus under formation.

Scale 14 : Total length of fish :
768.35 mm.
Date of collection :
10-7-1955
Shows already formed annulus

Scale 15 : Total length of fish :
322 mm.
Date of collection :
25-1-1954
Shows accessory checks which are misleading.

Scale 16 : Total length of fish :
844.5 mm.
Date of collection :
16-12-1952
Shows accessry checks which are misleading.

(Two lines, one above the other, at the extreme left lower margin of each print, show the total length of the actual corresponding scale at its antero-posterior axis in relation with the photograph arranged along the same axis.)

correlation. But, since in the present work, scales were picked from the same general location, a value of r of $+0.96$ is considered high enough to warrant the assumption of a rectilinear regression. In similar studies on certain species of the same family as that of *Mrigal* viz., *Labeo rohita* and *Catla catla*, where scales from identical locations were taken, a value of r of $+0.99$ was found in either case (Jhingran, *unpublished*). Further, flexions usually occur in the early growth of the rudimentary scale in the body-scale relationship. The portion of the scale representing growth from the time of the first annulus formation to death, appears to be nearly rectilinear in most populations which have been studied (Smith, 1955 ; Carlander, 1956). The correctness of the assumption of rectilinear relationship between scale and body length of *Mrigal* is further borne out by the general agreement of the results obtained by the growth study of this fish by two alternative methods of inquiry, which are completely independent of each other, and are presented in the following sections of this paper.

Plates V to VII show eleven photographs of *Mrigal* scales taken from fish, the total lengths of which are specified in the individual illustration. Photographs 1—10 show a varying number of annuli as specified in the legend. Small conical paper strips, indicating positions of the annuli, have been pasted on the photographs to show the exact markings interpreted as annuli. The illustrated scales are taken from fish of varying lengths, and show, in general, increasing number of annuli with increase of fish length, which phenomenon is regarded as a preliminary evidence substantiating the validity of regarding these markings as annular in nature. Another evidence pointing to the same end is the decreasing distance, seen in the majority of the illustrated scales, between successive annuli with the advancement of age, an indication of progressively slow rate of growth with increase

TABLE 1

Size-frequency distribution of Mrigal *at various ages revealed by scale study*

Class centres in millimetres	Number of completed annuli											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
137	9											
182	75											
227	123											
272	203	1										
317	145	3										
362	125	20										
407	55	54										
452	10	92										
497	3	113	10									
542	—	66	13									
587	—	59	25									
632	—	38	28	6								
677	—	20	36	7								
722	—	8	32	8	3							
767	—	2	30	22	6	1	1					
812	—	—	14	31	8	3	—					
857	—	—	1	27	30	17	2	1				
902	—	—	—	10	26	19	17	10				
947	—	—	—	—	3	11	7	5	2			
992	—	—	—	—	—	—	2	2	4	3	3	
1037	—	—	—	—	—	—	—	—	2	1	1	2
Total	748	476	189	111	76	51	29	18	8	4	4	2
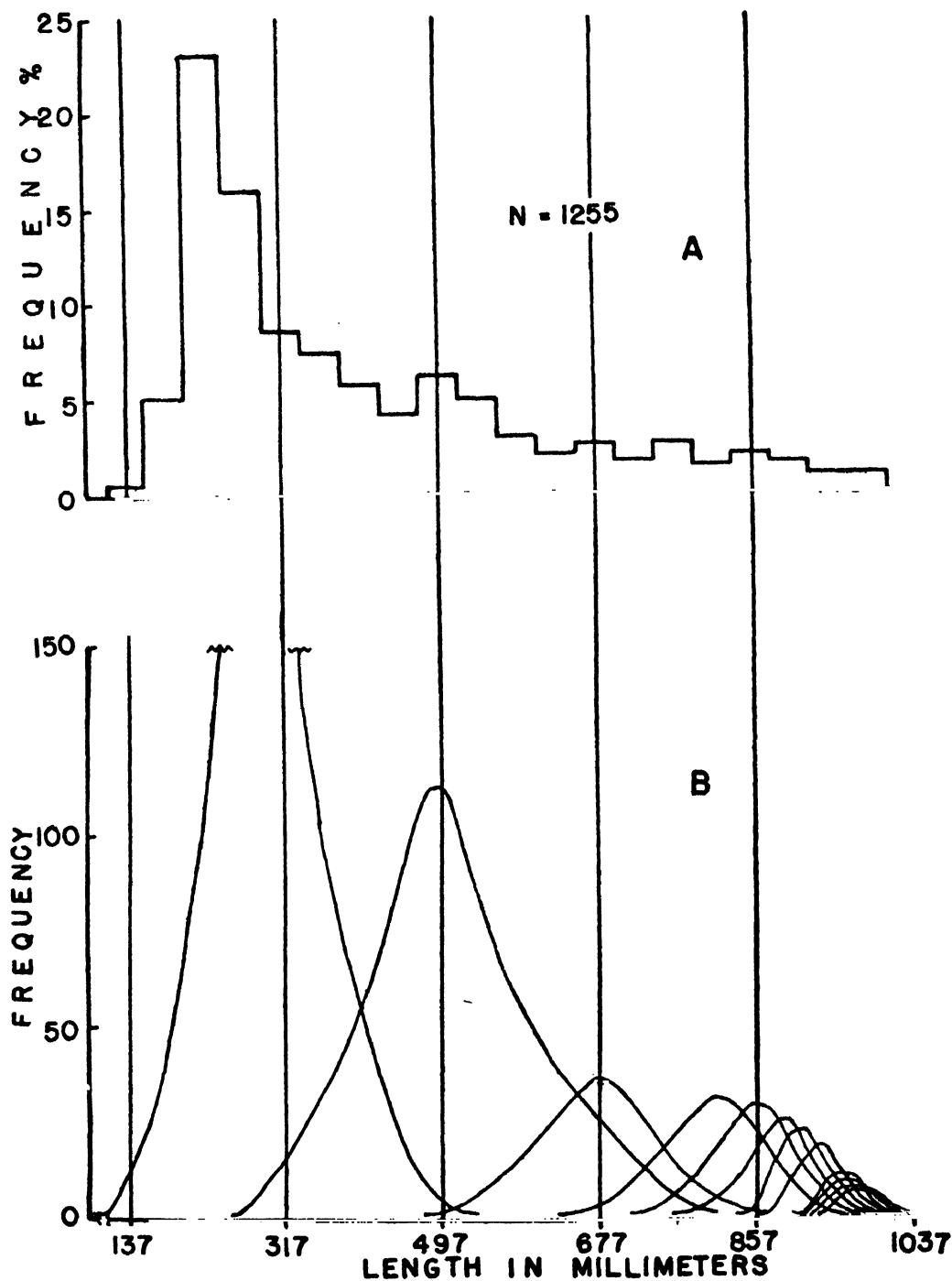
TEXT-FIG. 2.

Comparison of sizes attained by *Mrigal* at the time of various annuli formation (B) with themodes observed in the length-frequency distribution (A).

in age.   A close view of one of the scales, more magnified than the others, illustra-
ted in photograph 12 (Plate VIII), shows this phenomenon with exceptional clarity.
While majority of the scales of *Mrigal* (except the regenerated ones) showed easily
interpretable markings, some presented difficulties, and these were, therefore, not
utilised for back calculation purposes.   Two such, for the time being, inexplicable
scales, showing annuli-like markings very close together are shown in photographs
15 and 16 (Plate VIII).

Table 1 presents a frequency distribution of the sizes of *Mrigal* attained at the
end of the first twelve years of its life, based on back calculations of observations
on the locations of annuli on scales from 807 specimens of the size range : 103.0 mm.
to 1,010.0 mm.   Figure 2B shows a visually smoothened plot of this frequency
distribution.   Emergence of a frequency, by this procedure, resembling normal
distribution, is regarded as one of the proofs of the validity of the scale method
as applied to *Mrigal*.   If annuli are formed irregularly, without any temporal or
spatial relationship to age, such a normal, or near normal, frequency distribution
of the lengths, centred round each age, is not likely to occur.

While the peripheral annuli in scales showing more than four rings were found
crowded together, in younger specimens the outermost annulus was found close
to, or at the margin of the scales, in samples collected during the period : March-
June :   Scale 13 (Plate VIII) illustrates a marginal annulus on a scale from a fish
captured in April and scale 14 an annulus near scale margin already formed by
July.   From such observations it has been tentatively inferred that the annuli
are laid down during the spring or summer months (March-June), the probable
reasons for which are stated later in this paper.   Table 2 gives pooled size-frequency
distribution obtained in the months : March to May or March-June separately
for the years, 1953-1956 and also the total pooled frequency for these months.
Figure 2A presents a histogram of the total pooled size-frequency distribution of
Mrigal for the spring and summer months (March—June) of the years : 1953-1956,
"superimposed" on a smoothened plot of the frequency distribution of sizes attained
at various ages worked out by utilisation of scales for age and growth study, referred
to earlier.   Figure 2A shows one positively skewed prominent peak located at
the extreme left in 228 mm. class followed by a less prominent one in the 497 mm.
class and three others which are poorly indicated in 678, 768 and 858 mm. classes.*
The position of the first peak in 1953 and 1956 (Table 2) data is in 228 mm. class,
both showing positive skewness, and in 1954 in 273 mm. class.   Figure 2B shows
the positions of the first five peaks in 273, 497, 677, 812, and 857 millimetre
classes.   A general similarity in figures 2A and 2B with a close correspondence in
the positions of the peaks in the two, in particular, is regarded as a strong evidence
demonstrating that the markings interpreted as annuli are perhaps really so.   The
ill-defined nature of the last three modes in figure 2A is partly attributable to a
greater dispersion in the sizes of *Mrigal* with increase in age, resulting in great
overlap of successive year classes and partly, it is believed, to a high fishing
mortality which the fishery has undergone for generations, so that, at the present
day, the bulk of the catches consists of juveniles.

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* Owing to slightly different seriation schemes having been followed in the preparation
of Tables 1 and 2 there is a difference of one millimetre in the corresponding classes of these
two tables, which is also reflected in Figure 2.   It is obvious that such a slight difference
does not at all affect the comparisons made in Figure 2.

TABLE 2

Percentage of pooled size-frequency distribution in spring and summer months during the years 1953-1956

Class centres in millimetres	1953 March–May	1954 March–May	1955 March–June	1956 March–June	Total pooled frequency
3					
48					
93					
138	0.3	2.4		0.2	0.6
183	4.0	15.8	0.3	3.9	5.1
228	39.0	22.5	—	27.8	23.0
273	14.3	31.1	—	19.4	15.9
318	18.4	7.6	3.2	6.6	8.5
363	8.1	5.7	11.2	5.3	7.3
408	1.1	3.3	14.7	4.1	5.7
453	0.3	2.4	10.5	3.5	4.2
498	1.1	3.3	14.4	5.5	6.3
543	0.4	1.0	13.0	4.5	4.9
588	0.4	1.4	6.3	2.9	2.9
633	0.4	—	4.6	2.3	2.0
678	1.5	—	4.6	2.9	2.5
723	—	1.0	3.9	1.8	1.8
768	1.5	0.5	3.9	3.3	2.6
813	1.1	—	2.1	1.6	1.4
858	4.0	0.5	1.0	2.0	2.0
903	2.2	—	2.1	1.6	1.6
948	1.5	0.5	1.4	0.6	0.9
993	0.4	1.0	2.8	0.2	0.9
1038					
No. of specimens in sample	272	209	285	489	1255

TABLE 3

Average size and weight of Cirrhina mrigala *at the time of various annuli formation*

Number of completed annuli	Total length in		Weight in	
	Millimetres	Inches	Grams.	Pounds
(1)	(2)	(3)	(4)	(5)
I	290.9	11.5	245.7	0.54
II	511.4	20.1	1,512.0	3.33
III	670.5	26.4	3,618.0	7.98
IV	797.4	31.4	6,324.0	13.94
V	858.8	33.8	8,030.0	17.70
VI	888.5	35.0	8,960.0	19.75
VII	911.0	35.9	9,712.0	21.41
VIII	921.8	36.3	10,090.0	22.24
IX	947.0	37.3	11,000.0	24.24
X	958.25	37.7	11,430.0	25.20
XI	958.25	37.7	11,430.0	25.20
XII	992.0	39.1	12,770.0	28.15

The poor representation of large sized fish in the catches is also, obvious in general from figure 2.

AGE AND GROWTH IN LENGTH

A. *As interpreted from scale study.*—The data presented in Table 1 have been utilised to determine the mean lengths of *Mrigal* attained in the first twelve years of its life, which are shown in columns 2 and 3 of Table 3 in millimetres and inches respectively.   Figure 3 shows the absolute growth curve of this species representing the average sizes of *Mrigal* at the time of the formation of the successive twelve
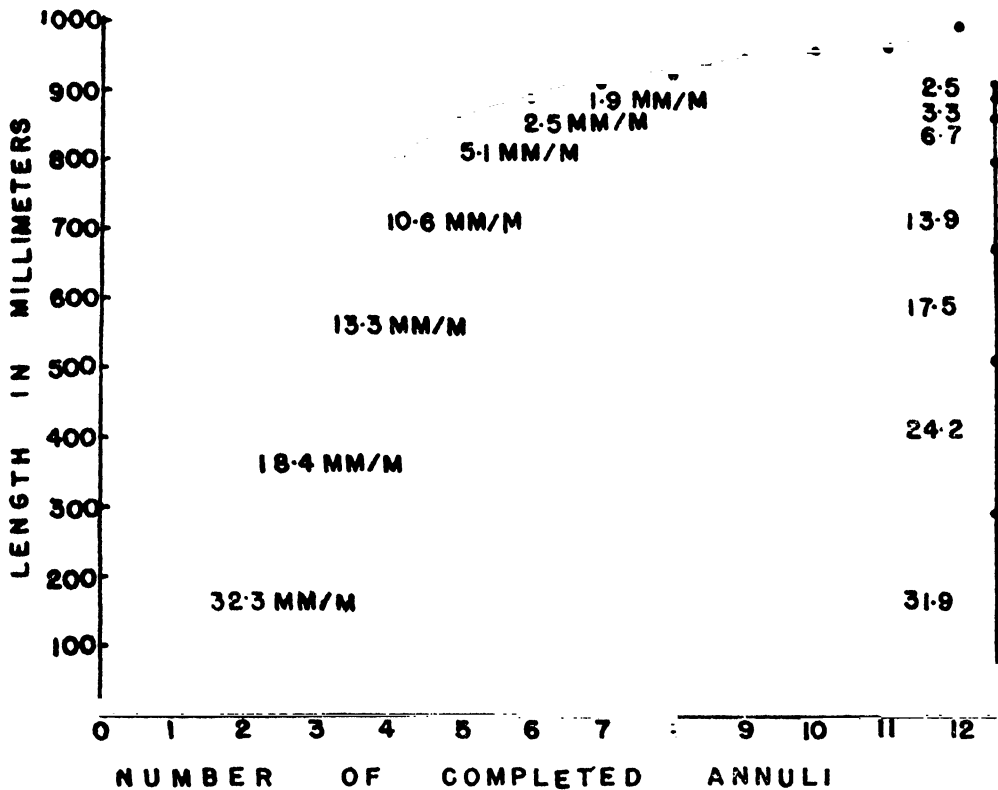
TEXT-FIG. 3.

The curve of growth of *Mrigal* in length in the first twelve years of its life showing also the rate of growth during the first seven years and the percentage of the total of seven years' growth occurring during each individual year.

annuli.   Occurrence of a very rapid growth in length is indicated in the first four years of its life followed by a period of slow growth in the following three years. From the end of the seventh year onwards, till as far as known, the growth may be categorised as very slow.   The growth increments in length and the growth rate in millimetres per month calculated separately for each of the seven years of life, are given in columns 2 and 3 of Table 4.   The latter is also indicated at relevant positions in Figure 3.   A progressively declining growth rate with advancement of time is clearly seen in Table 4, and also in Figure 3.   The maximum size attained

by *Mrigal* has been theoretically deduced to be 1,039 mm (see section C.). 87.7 per cent of the total growth of this fish in length is covered in the first seven years of its life. 31.9, 24.2, 17.5 and 13.9 per cent of growth take place in the first four years respectively (out of a total of seven years). The relative growth in length during each year (out of a total of seven years) is tabled in column 4 of Table 4, and the extreme right portion of Figure 3 illustrates the percentages graphically along with the growth curve.

TABLE 4

Annual growth increments and growth rate/month of Cirrhina mrigala in length and weight in its first seven years

Duration between checks	Growth in length			Growth in weight		
	Growth increment in mm.	Growth rate in mm. per month	Percentage of total growth (Relative growth)	Growth increment in grams	Growth rate in grams per month	Percentage of total growth (Relative growth)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0 – I	290.9	32.3	31.9	245.7	27.3	2.5
I – II	220.5	18.4	24.2	1266.3	105.5	13.0
II – III	159.1	13.3	17.5	2106.0	175.5	21.7
III – IV	126.9	10.6	13.9	2706.0	225.5	27.9
IV – V	61.4	5.1	6.7	1706.0	142.2	17.6
· – VI	29.7	2.5	3.3	930.0	77.5	9.6
VI – VII	22.5	1.9	2.5	752.0	62.7	7.7
Total	911.0		100.0	9712.0		100.0

B. *Growth a. interpreted from size frequency study.*—While the study of *Mrigal* sca'es has furnished an overall picture of the growth of the fish, a study of the length-frequency distribution,   data for which are available for about four years besides corroborating the picture of early growth of this species delineated by scales, throws additional light on the details of the growth rate in the first three years of its life. Figure 4 shows the histograms of the length frequency distribution for the various months, over the period, June 1952 to June 1956, which were necessary to be able to trace the modes and also for elucidating the picture of growth. *Mrigal* is known to breed in North India during the monsoon months, June-August, and the first time 'the fish of the year' enter the commercial fishery as fingerlings during the month of September. However, to complete the picture, collections of the fry of the fish were made in June-July 1952, with a net made of mosquito netting. The *Mrigal* fry usually occurs mixed with those of some allied genera, collectively called major carps. Length measurement of 100 (mixed collections showing very little size variation) were taken, and included in the length-frequency data for that month. In the following account the term Group zero is used to designate the fish of the year, that is, fish which are in the same calender year as their year of hatching ; Group one for fish which are in their second calendar year, Group two for fish which are in third calendar year and so on.

Analysis of 1952 data.—Four collections were made during the period, June-December. The modes at 3 mm. class in June–July, at 138 mm. class in September-October and at 183 mm. class in December are caused by the fish of the year and represent the zero group. The peak centred at approximately 408 mm. class in June–July and the isolated peak at 363 mm class in November are interpreted as representing the first year group and that at 543 mm. class in June–July and 633 mm. class in December as representing the second year group. The right hand "tail" in June–July, 1952, and the rather prominent group in the class ranges centred from 813 mm. to 1,038 mm., in November, are undoubtedly caused by fish highly heterogenous in age and represent a great overlap in sizes of fish varying in age from 3 to 12 years or more. The reasonableness of the interpretation presented here is seen when pooled data for different years are examined, which has been done and mentioned at a later point in this paper. The lack of representation of even younger size groups during all the months is attributable to selectivity of gear or probable segregation of year classes or migration to areas either unfished or inaccessible to gear. Lack of progress in modes, with the passage of time is attributed to errors of random sampling.

Analysis of 1953 data.—Nine collections were made during the course of 1953. The prominent modes at the extreme left in September, November and December are caused by the fish of zero group. Group one appears in March, April, May, July, September, October and November in 273, 318, 288, 363, 363, 318 and 453 mm. classes respectively. The peaks in 498 mm. class in March and in 588 mm. class in November appear to be caused by fish of group two. The groups, spread from class centres 768 to 993, in January and March, are caused by a heterogenous assemblage of various year classes.

Analysis of 1954 data.—Nine collections were made in the year 1954. The peaks in 138 mm. class in October and November represent zero group of 1954, Group one is well represented by modes in seven collections of the year viz., January-February, March, April-May, June, July, August, and October, centred in 273, 228, 273, 273, 363, 408, and 453 mm. classes respectively. Indications of Group two occur in 498 mm. class in March, in 588 mm. class in October and in 633 mm. class in December. Group three appears to be represented at 687 mm in January-February.

Analysis of 1955 data.—Eleven collections were made in the year 1955. A series of modes at the extreme left of the histograms for September, October, November and December prominently represents fish of that year. Fish of Group one appear very insignificantly in 228 mm. class in January. Group two is represented by modes in 498 mm. class in March, 453 mm. class in April, 408 mm. class in May, 543 mm. class in June and July and in 498 mm. class in September. Group three appears to be represented in 687 mm. class in February-March and in 768 mm. class in July.

Analysis of 1956 data.—Six collections were made upto June in 1956. Group one is seen in modes within 183 mm. class in January and 228 mm. class in March, April and June and Group two in modes within 453 mm. class in January and 498 mm. class in March. Group three is detectable in 633 mm. class in January and in the same class in February and May.

Threading together piecemeal evidences on the lengths of younger age groups in various months, suggested by relatively better defined modal locations (given in Table 5) in the thirtynine collections, spread over four years, a picture of the early growth of *Mrigal*, carrying it upto about the middle of the second year group (third calendar year of fish's life) has been drawn in figure 5. Due to a combination of probable causes, already stated, even all the younger age groups are hardly represented in the catches of a given month, which make the interpretation of length-frequency data very difficult. (The collections of June–July, 1952, October, 1954 and January, 1956, are relatively better representative of the year

classes.) The picture is, however, brightened considerably if the data for the corresponding months of all the four years are pooled, although such a procedure ignores
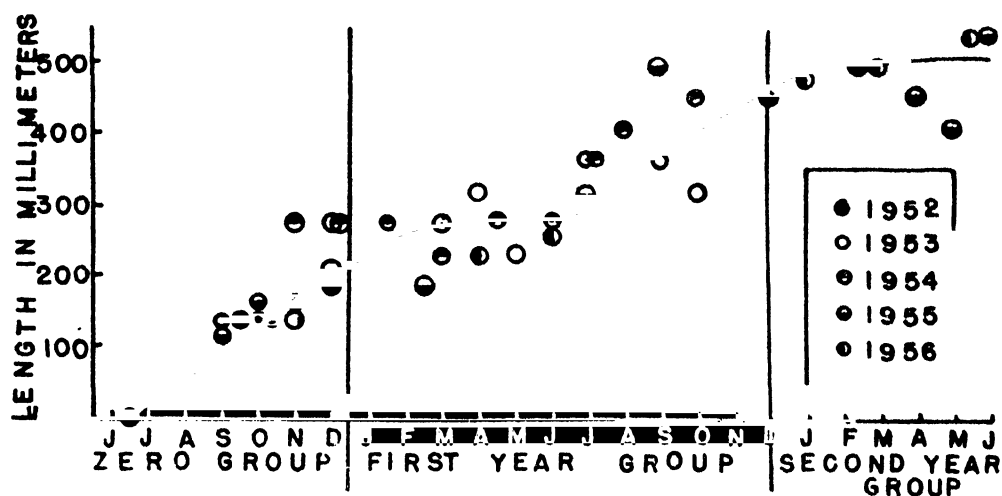
TEXT-FIG. 5.

Growth curve of *Mrigal* interpreted by the length-frequency data shown in Figure 4.

TABLE 5

Approximate modal locations in millimetres utilised to draw early growth picture upto about 2 year old stage of Mrigal

1952 month : mid-point		1953 month : mid-point		1954 month : mid-point		1955 month : mid-point		1956 month : mid-point	
zero-year group									
June-July	3	—	—	—	—	—	—	—	—
Sept.-Oct.	138	Sept.	138	Oct.	138	Sept.	138	—	—
—	—	Oct.	138	—	—	Oct.	138	—	—
—	—	Nov.	138	Nov.	138	Nov.	138	—	—
Dec.	183	Dec.	273	Dec.	228	Dec.	183	—	—
first-year group									
—	—	—	—	Jan.-Feb.	273	Jan.	228	—	—
—	—	March	273	March	228	—	—	—	—
—	—	April	318	—	—	—	—	April	228
—	—	May	228	April-May	273	—	—	—	—
—	—	—	—	June	273	—	—	June	228
June-July	408	July	363	July	363	—	—	—	—
—	—	—	—	Aug.	408	—	—	—	—
—	—	Spet.	363	—	—	—	—	—	—
—	—	Oct.	318	Oct.	453	—	—	—	—
second-year group									
—	—	—	—	—	—	—	—	Jan.	453
—	—	March	498	—	—	Feb.-March	498	March	498
—	—	—	—	—	—	April	453	—	—
—	—	—	—	—	—	May	408	—	—
—	—	—	—	—	—	June	543	June	543
—	—	—	—	—	—	Sept.	498	—	—
Dec.	633	—	—	—	—	—	—	—	—

the variations in the growth rates which might be occurring from year to year. Table 6 presents such a pooled size-frequency distribution and Figure 6 illustrates the histograms for all the twelve months of the year. Although the breeding of *Mrigal* is known to extend from June to August, July has been taken as a typical month for breeding and an area enclosed with dashes at the extreme left of the histogram for July in 3 mm class indicates the recruitment of the species into the environment though not into the fishery at this stage. The growth of the fry is extremely rapid during the monsoon months, and by autumn they reach the fingerling stage indicated by modes in 138 millimetre class in September, October and

TABLE 6

Percentages of pooled size-frequency distribution of the corresponding months during the period June, 1952 to July, 1956

Class centres in millimetres	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
3												
48												
93									18.2	13.2	4.4	
138	7.7	7.7				1.0			55.4	60.2	43.7	2.9
183	16.0	15.0	10.6	0.9	3.5	4.8	0.8		9.9	8.5	17.5	18.5
228	12.4	13.9	26.7	16.4	46.2	11.9	2.6	0.9	6.5	3.0	4.0	14.8
273	15.4	16.4	8.4	23.9	8.9	14.6	3.1	1.3	4.8	2.5	6.7	23.4
318	4.0	5.5	0.9	14.2	5.8	7.3	9.0	18.9	1.4	2.3	5.5	4.2
363	2.3	1.8	2.7	17.0	4.4	9.3	23.0	25.0	1.8	3.9	2.9	1.5
408	5.0	1.8	4.0	6.9	4.9	9.0	20.4	25.0	1.1	3.8	1.3	1.0
453	8.0	2.5	6.2	7.5	2.2	4.4	10.3	6.9	1.3	4.2	0.2	1.3
498	4.0	3.6	12.0	4.7	3.1	4.9	2.8	2.6	2.2	1.7	1.3	1.1
543	4.3	4.7	6.2	2.5	1.8	7.8	5.2	6.9		1.9	1.7	4.3
588	3.0	5.1	3.5	1.9	2.7	4.6	3.4	2.6	0.2	1.8	2.9	6.4
633	6.0	5.1	2.2	0.3	3.5	2.0	3.1	1.7	0.3	1.7	0.7	7.8
678	4.7	8.8	2.2	1.3	3.5	1.9	1.3	0.9	0.2	0.5	0.7	6.4
723	3.7	4.4	0.9	0.3	1.8	2.6	1.3		0.5	0.5	0.3	3.3
768	1.4	0.7	2.2	1.3	1.8	4.1	4.1	2.6	0.2	0.2		1.4
813	0.4	0.4	1.8	0.6	0.4	2.0	2.6	1.7		0.1	0.7	0.1
858		0.4	3.5		3.5	2.4	2.6				0.4	0.6
903	0.7	0.7	1.3		1.8	3.2	2.6				2.4	0.4
948	1.0	0.4	2.6			1.3	1.0				2.0	0.4
993		0.1	2.2	0.3		0.9	0.8				1.6	0.1
1038			0.5								0.2	
No. of specimens in sample	299	274	226	318	225	589	387	116	626	950	451	718

= 5179

November histograms in Figure 6. Breeding, spread over 2½ or 3 months, may be responsible for non-shifting of the modes during these three autumn months inspite of which, however, the average length of the size group shows a progress during these months as may be seen in the negatively skewed distribution of the relevant size-group in September changing to positive skewness in November. Prolonged breeding, however, shows its effect in the great spread of the length range of this group, as is seen in the December histogram of figure 6, where the fish of the zero group exhibit a bi-modal distribution with the approximate centre in the 228 mm class. Group one can be traced by prominent modes in all the histograms from January to August, and it appears that progress of average lengths during the period—January-February to May-June—is slow, and becomes rapid thereafter. September to November histograms do not show any age group prominently except that of the zero group: this is due to the great concentration of the energies
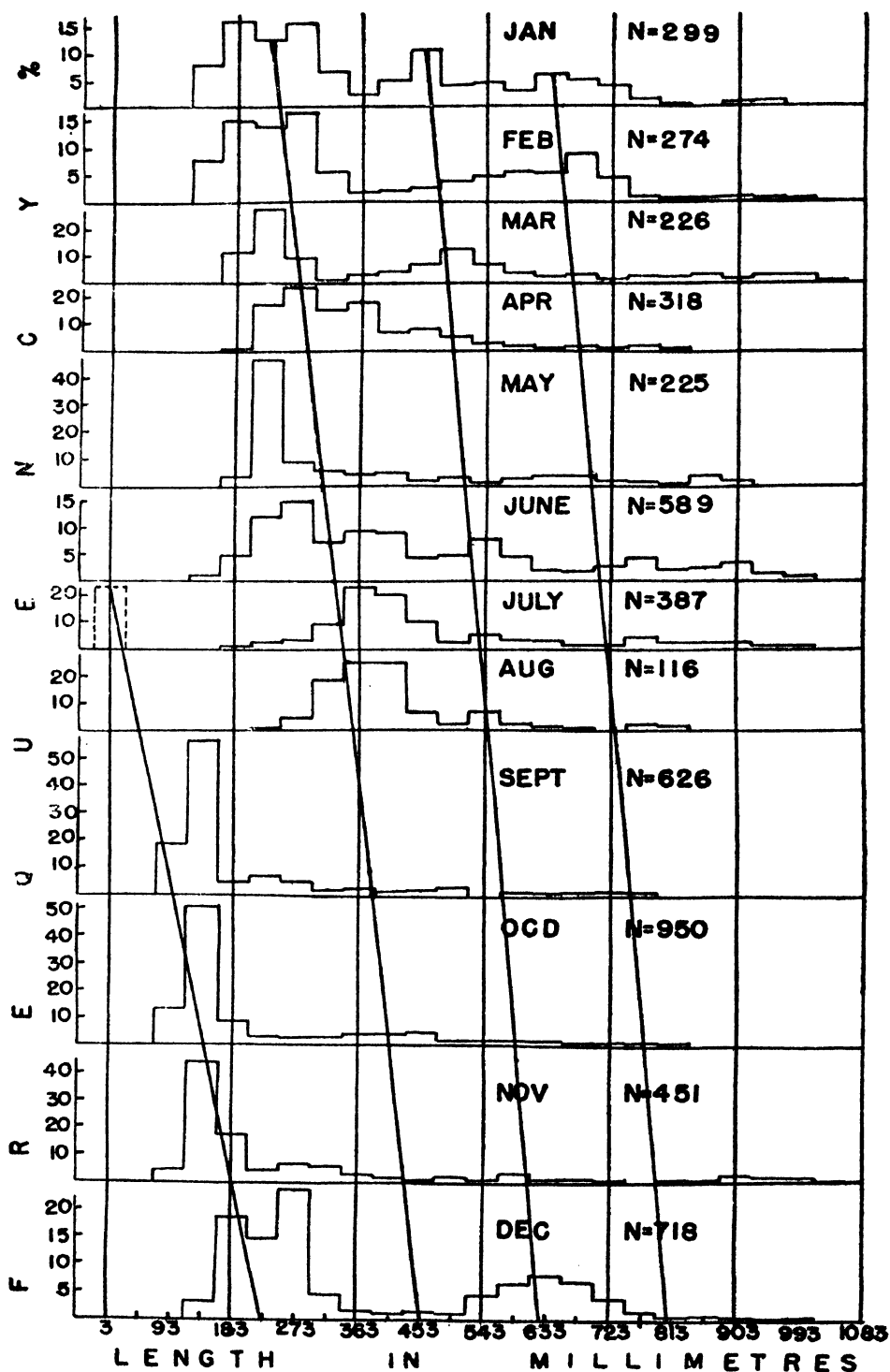
TEXT-FIG. 6.

Histograms of the length-frequency distribution of *Mrigal* of the data—June, 1952 to June, 1956—Pooled for corresponding months given ia Table 6.

of fishermen to the destructive capture of carp fingerlings (Jhingran and Chakra-borty, 1958) and capture of *Hilsa*, which is abundant at this time of the year (Jhingran, 1956) in the River Ganga at Buxar. The absence of fish of Group one, in the histogram for December, where a mode centred in the 633 millimeter class is present, is inexplicable specially when this group is so prominently seen in the 453 mm class in January in the histogram for which month it is to be termed as fish of Group two. Group two is traceable in January, March, April and May in 453, 498, 453 and 498 millimetre classes respectively, in June-August in 543 mm class and in November and December in 588 and 633 mm classes respectively. Group three is traceable in 633 mm class in January and in 687 mm class in February, March and April. Slanting lines passing through or the vicinity of modes, repre-senting a somewhat simplified picture of growth and tracing it upto the third year group stages have been drawn in Figure 6. The parallel trend of the straight lines indicates the correctness of the interpretation of growth offered here. Pooling of length-frequency data has rendered the carrying of growth picture to a measure farther than what was possible in the interpretation of unpooled length-frequencies. The positions of modes discernible in Figure 6 are given in Table 7 enabling a detailed

TABLE 7

Approximate modal locations in millimetres in the pooled size-frequency distribution data utilised to draw early growth picture upto about Group 3 stage of Mrigal

Month : Mid-point zero-year group		Month : Mid-point first-year group		Month : Mid-point second-year group		Month : Mid-point third-year group	
----	----	Jan.	183	Jan.	453	Jan.	633
----	----	Feb.	273	--	--	Feb.	678
---	---	March	228	March	498	March	678
---	--	April	273	April	453	April	678
---	---	May	228	May	498	--	---
June-July	3	June	273	June	543	---	---
---	---	July	363	July	543	---	---
---	---	August	363	August	543	---	---
Sept.	138	---	---	---	---	---	---
Oct.	138	Oct.	453	---	---	---	---
Nov.	138	---	---	Nov.	588	---	---
Dec.	273	---	---	Dec.	633	---	---

interpretation of the histograms in the shape of a growth curve shown in Figure 7. There is no appreciable difference between the growth pictures elucidated by scales and those brought out by the use of the length-frequency distribution, which again shows the correctness of the interpretation given here. The growth curve drawn by passing a line through the middle of scatter of plot of modal locations (Figures 5 and 7) throws light on the relative rates of growth in different seasons of the year. Rainy and autumn seasons (July-October) are those of fast growth, additional support for which phenomenon is obtained by the general observation of great abundance of planktonic organisms during these months. The food of fry and fingerlings of major carps is known to consist of plankton largely. Spring months leading into summer are interpreted as periods of slow growth. It is during these months roughly that the annuli are believed to be laid. Investigations on the feeding of *Mrigal*, extending over a full year, carried out by another worker of this sub-station have shown that in *Mrigal*, of all sizes, the intensity of feeding is very low during spring and summer months. Starvation thus suggests itself as a probable cause of annulus formation. That the growth during these months is merely slowed down, but does not cease completely, is borne out by the large

broad band-shaped annuli shown in practically all the illustrations given in plates I to IV. It may be seen with exceptional clarity in scale 14 (plate IV). The growth curve thus merely tends to become horizontal during these months but does not become parallel to the x-axis (Figures 5 and 7).
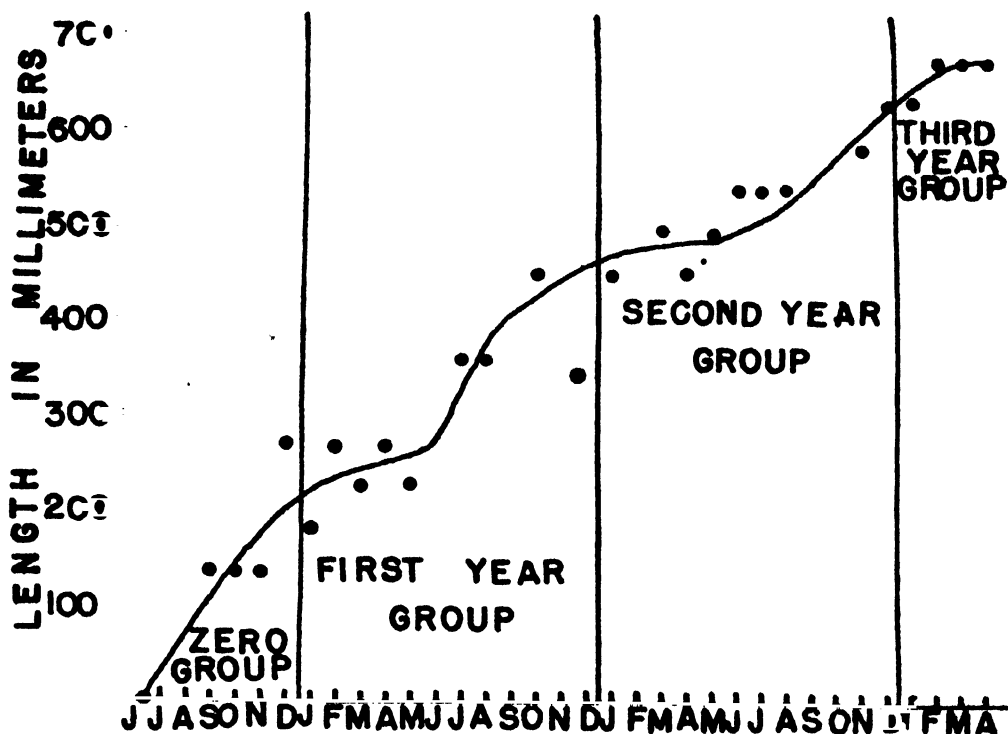
TEXT-FIG. 7.

Growth curve of *Mrigal* interpreted by pooled length-frequency data graphically shown in Figure 6.

Because of the crowding of annuli of later years towards the scale margin reading of their exact locations became difficult. This fact has resulted in the back calculations of sizes, in some cases, also defective. Same average lengths emerge for the tenth and the eleventh years which obviously cannot be the case because the fish grows to over 1,000 mm. Similarly the point for twelfth year is off the line in Figure 3. Further, out of a theoretical maximum length of 1,039 mm. only 992 mm. are accountable with the material studied, and this fact enables the conclusion to be drawn that *Mrigal* attains an age higher than twelve years. Examination of more scales of large-sized fish and use of scale enlarging apparatus may throw more conclusive light on the growth of this fish during the period preceding natural death due to old age. These are, however, largely theoretical matters because practical fishery considerations cease in this fishery after an attainment of the age of seven.

C. *Maximum length attained by Mrigal*

The ultimate length attained by *Mrigal* has been determined graphically at a point when the length at age n equals length at age $n+1$. Figure 8 shows a

3

plot of sizes at age *n* against sizes at *n+1* wherein an intercept of a line at 45° is shown with the transformed growth line.

Mathematically : $l_\infty = \dfrac{l_1}{l-k}$ where l_∞ = ultimate length, l_1 = length at age *I*

and $k = \dfrac{l_3-l_2}{l_2-l_1}$ or $\dfrac{l_4-l_3}{l_3-l_2}$ or $\dfrac{l_5-l_4}{l_4-l_3}$ or $\dfrac{l_n-l_{n-1}}{l_{n-1}-l_{n-2}}$

In the case of *Mrigal* l_∞, or, the ultimate length, works out to be 1,039 millimetres, as may be seen in Figure 8. The maximum length of *Mrigal* encountered in the field was 1,016 mm on 18th November, 1952, only 23 mm short of the theoretical ultimate length. This fact shows that the collections which have been examined in the present study, cover practically all the fish sizes which occur in the population sampled.
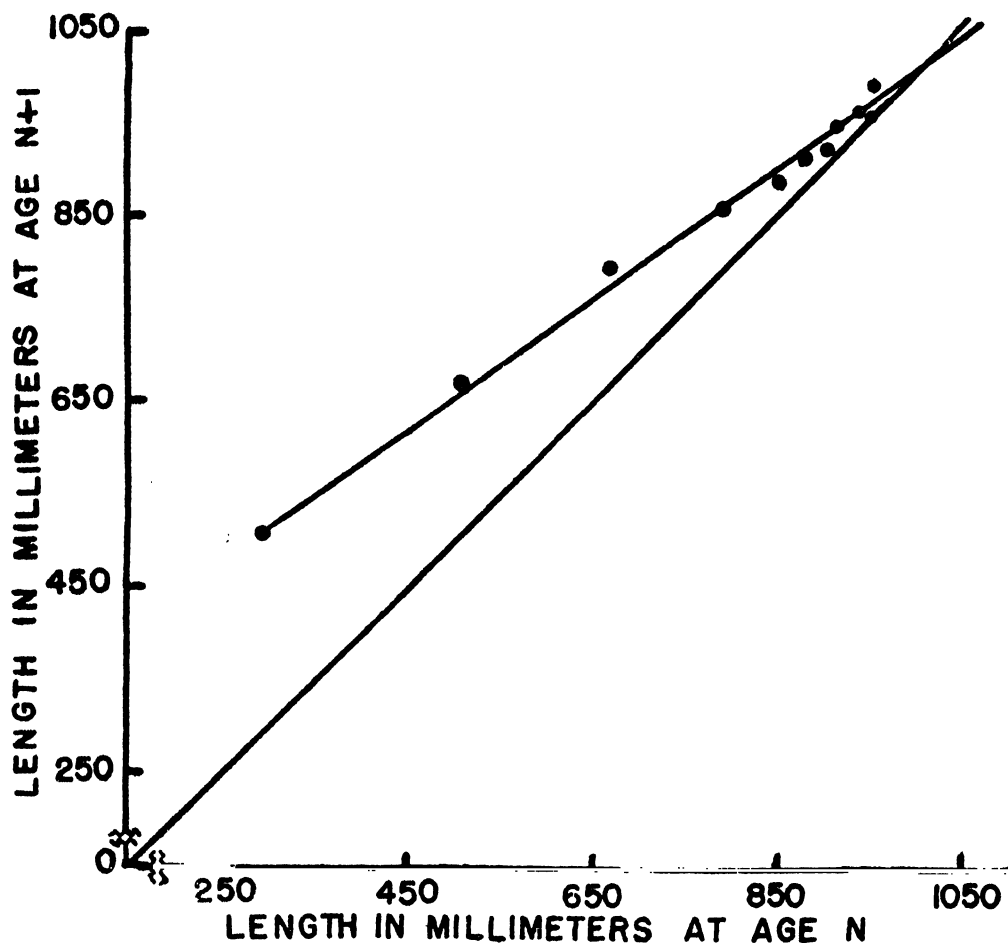
TEXT-FIG. 8.

A plot of sizes of *Mrigal* at age n against sizes at age n+1 showing the ultimate length attained by the fish.

AGE AND GROWTH IN WEIGHT

An attempt was made to find out whether the weight-frequency distribution lends further support towards validating the application of scale-method in *Mrigal*, and whether the same can be successfully utilised for the study of growth in weight of this species. Weight of *Mrigal* sampled for the present study, ranged from 3.5 gm. to 17,548.6 gm., and Figure 9 presents the weight-frequency distribution in 71 classes with a class interval of 250 gm. The number of specimens beyond the 4,875.5 gm. class are very few to elicit any clear modes and even those in smaller classes are rather difficult to interpret. A class interval of 250 gm. appears too wide to bring out peaks of fish representing groups zero and one. Apparently for this reason the first peak in the histograms, for all the twelve months of the year, lies very close to the y-axis and hardly shows progress with the advance of time. In order, therefore, to elucidate growth in weight of fish of zero year class, the class interval was reduced to 50 gm. and specimens weighting upto 1 kg. seriated, combining data of the corresponding months over the duration—June, 1952 to July, 1956. Table 8 shows such data. When thus treated the modes representing fish of the year and upto the completion of the second calendar year become clearer, as are shown in Figure 10. The broad bases of the peaks in September, October, November, April and June and even bi-modality (situated close to each other) in December, January and February are interpreted to be due to somewhat prolonged breeding season of the species. Straight lines, passing through the approximate modal locations, making allowance for errors of random sampling,

TABLE 8

Percentage of pooled weight-frequency distribution of the corresponding months during the period June, 1952 to July, 1956 up to 1 kg. weight

Class range	Jan.	Feb.	March	April	May	June	July	Aug.	Sept	Oct.	Nov.	Dec.
− 50	23.7	27.3		0.3		0.8			82.0	75.0	71.8	21.3
51–100	16.7	14.9	12.7	11.4	7.9	12.0	2.9		4.6	6.2	6.9	12.4
101–150	20.0	23.8	64.7	12.9	7.9	15.1	1.6		4.1	1.5	2.1	24.3
151–200	13.7	15.5	7.2	16.4	7.9	16.8	1.2	1.1	2.9	6.8	3.3	23.8
201–250	7.1	8.3	1.2	9.3		10.6	5.7	7.8	1.2	1.5	4.9	8.9
251–300	2.0	2.4	1.2	6.8	2.6	6.8	4.9	17.8	1.3	1.2	3.1	3.1
301–350	1.0	3.6		2.9	5.3	2.3	4.1	3.3	0.3	0.9	2.3	0.9
351–400	1.0	0.6	0.4	7.5		3.1	3.3	7.8	0.2	1.4	1.0	0.9
401–450	0.5		0.4	8.6		5.1	5.7	1.1	0.2	0.9	0.5	1.3
451–550		1.2		7.5	7.9	3.1	17.8	3.3	1.3	0.8	0.8	0.2
551–600		0.6			10.5	0.8	0.8				0.5	
601–650	0.5		2.5	4.3		4.6	14.8	24.8		1.2	1.0	0.9
651–700	0.5	0.6			7.9	2.0	2.9					
701–750	4.2		2.1	4.6		5.4	12.3	8.9	0.8	2.4	0.3	0.9
751–800					18.4	0.6	1.2		0.9			
801–850	2.0		2.5	3.2		4.6	8.6	20.0		2.4		0.2
851–900					10.5	1.7	2.9				0.5	
901–950						0.8	0.8					
951–1000	7.1	1.2	5.1	4.3		3.5	8.6	4.4	0.7	3.8	1.0	0.9
					13.2	0.3	0.4					
No. of specimens in sample	198	168	235	280	38	350	244	90	588	860	390	449
												= 3890

are shown in Figure 10. The lines trace the weight of the zero and first year groups in approximate terms. The isolated peaks seen situated beyond the 275 gram class, in Figure 10, do not lend themselves to interpretation. Turning now
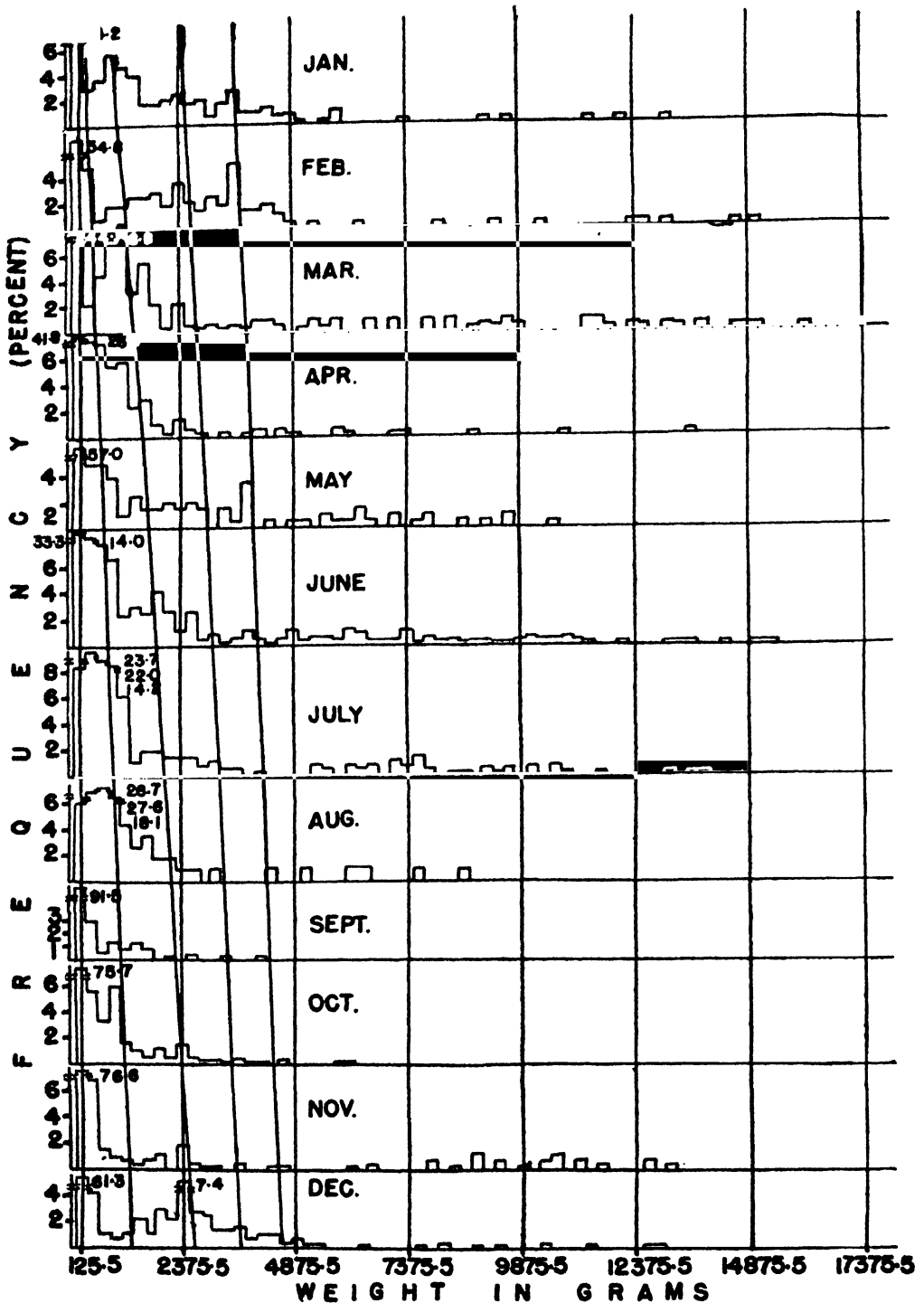
TEXT-FIG. 9.

Histograms of the weight-frequency distribution of *Mrigal* of the data—June, 1952 to June, 1956—pooled for the corresponding months. (The exact value of frequency is indicated near broken line marks in cases where it falls beyond the limit of the scale of y-axis.
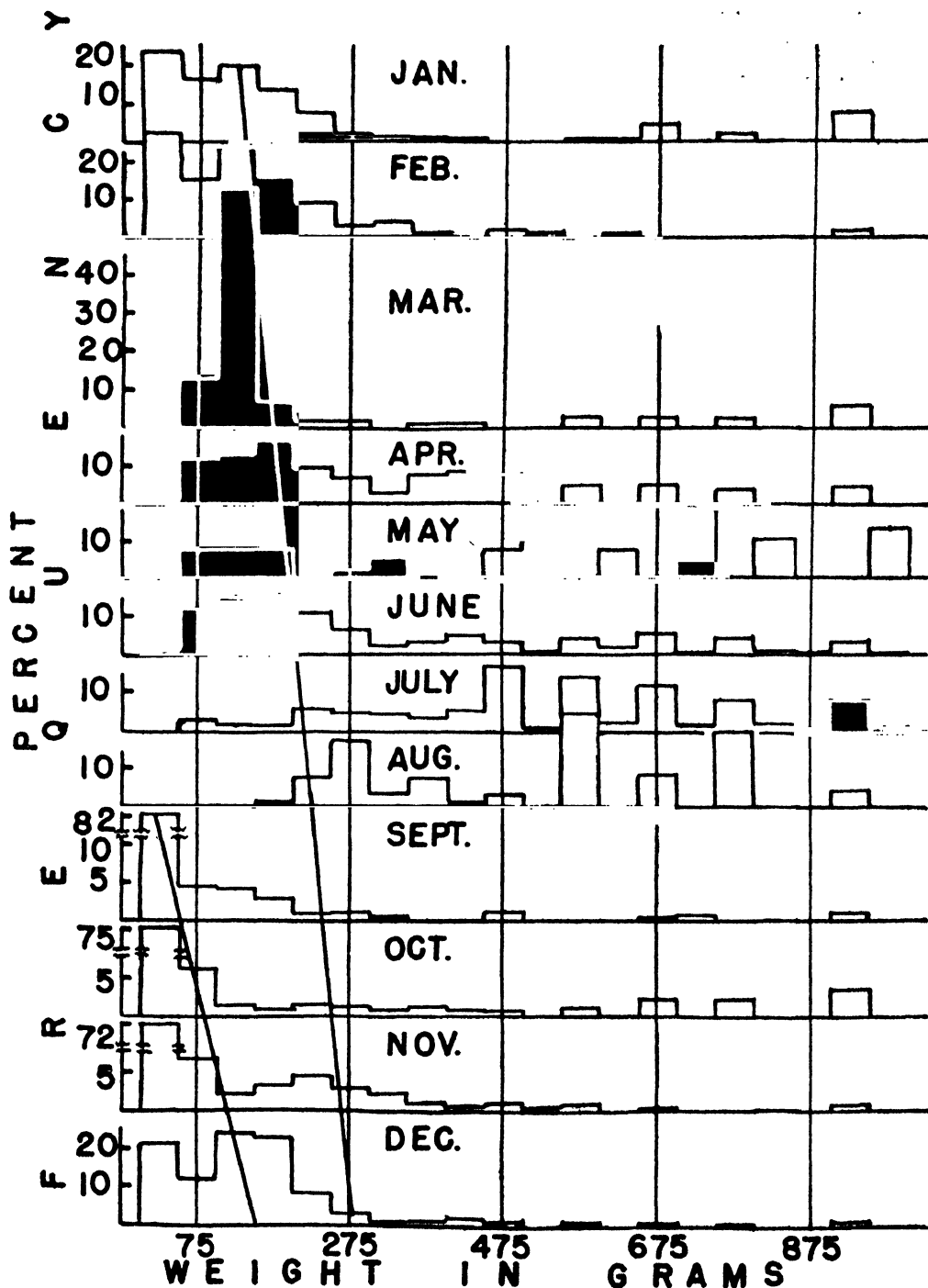
TEXT-FIG. 10.

Histograms of the weight-frequency distribution of *Mrigal* of fish weighing upto one kilogram given in Table 8. (The exact value of frequency is indicated near broken line marks where it falls beyond the limit of the scale of y-axis.)

to Figure 9, the first peak in all the histograms, lying close to y-axis, as stated above, represents combined peaks of zero and first year groups. However, the extension of the distribution of the first peaks towards right in general, with progress of time, and the occurrence of a distinct peak in 875 gm. class in October indicate the indentity of the first year group. Second year class is, on the whole, seen better isolated in Figure 9 and the peaks representing it are discernible in 875.5 gm. class in January, in 1,375.5 gm. class in April and 2375.5 gm. class in October, November and December. Indications of the third year class are seen in 2375 gm. class in the histograms from January to April and those of the fourth year class in 3625.5 gm. class in January and February and in 3825.5 gm. class in the histogram for May. The histogram for January shows four somewhat clear peaks in 125.5, 875.5, 2375.5 and 3625.5 gm. classes which approximately represent first to fourth year classes respectively.

On the whole it may be remarked that the weight-frequency distribution data do not lend themselves to analysis, as to growth study, very satisfactorily. Only an approximate idea of growth in weight, upto a little beyond the third year stage, can, however, be obtained from this study. Panels 4 and 5 of Table 3 give weights in grams and pounds respectively of fish of different ages determined by using the length-weight relationship formula specific for *Mrigal*. The growth in weight, as far as it can be ascertained from the weight-frequency study, agrees with lengths of fish of corresponding ages, and in this sense, the weight-frequency study adds support towards validating the application of scale-method to *Mrigal*.

Figure 11 shows the absolute growth curve of this species representing the weights of *Mrigal* at the time of the formation of the first twelve annuli. Sigmoid nature of the curve is obvious. The curve rises slowly at first, with an increasing slope, followed by a decreasing slope during the remainder of the curve. The growth increments in weight and the growth rate in grams per month, calculated separately for each of the first seven years of life, are given in columns 5 and 6 respectively of Table 4. The latter are inserted at appropriate positions in Figure 11. The growth rate rises from 27.3 gm. per month in the first year to 225.5 gm. per month in the fourth year of life. The point of inflection thus occurs when the growth rate in the fifth year decreases to 142.2 gm. per month and descends down to 62.7 gm. per month in the seventh year. 76.0 per cent of the total growth of this species in weight is covered in the first seven years of life, 65.1 per cent of which (of a total of seven years) occurs in the first four years. The relative growth in weight in each of the first seven years is given in column 7 of Table 4, and the extreme right portion of Figure 11 illustrates these percentages graphically along with the growth curve. Figure 12, which depicts a plot of percentage of total growth (total of seven years) in individual years in length and weight (given in columns 4 and 7 respectively of Table 4) against the appropriate number of completed annuli, brings out the difference in growth pattern between length and weight. While the maximum growth in length occurs in the first year, the maximum growth in weight takes place in the fourth year. The growth in length takes place at a progressively decreasing rate and that in weight gradually rises to a peak between the third and the fourth year, which period is preceded and followed by periods of slower growth rates.

Figures 9 and 10 also bring out another aspect of the *Mrigal* fishery quite lucidly, which is that fish of low weight dominate the catches. Without exception, the region of maximum frequency lies towards the extreme left of the histograms for all the months. Reference to this will be made again at a later stage in this paper.

A. *Size-Weight relationship*

Based on 4,930 observations on total lengths of fish varying from 78 mm. to 1,016 mm. and weights of corresponding fish ranging from 3.5 gm. to 17,548.6 gm.
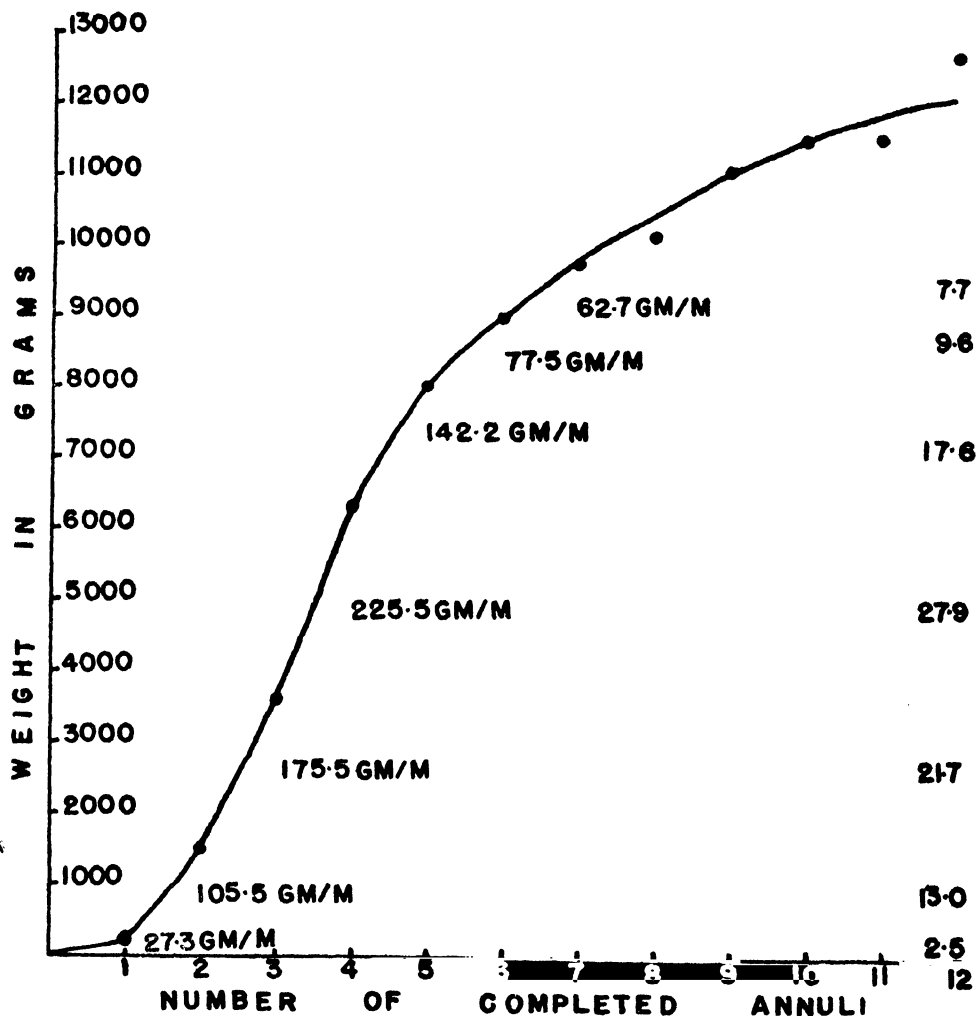
TEXT-FIG. 11.

The curve of growth of *Mrigal* in weight in the first twelve years of its life showing also the rate of growth in the first seven years and the percentage of the total growth of seven year occurring during each individual year.
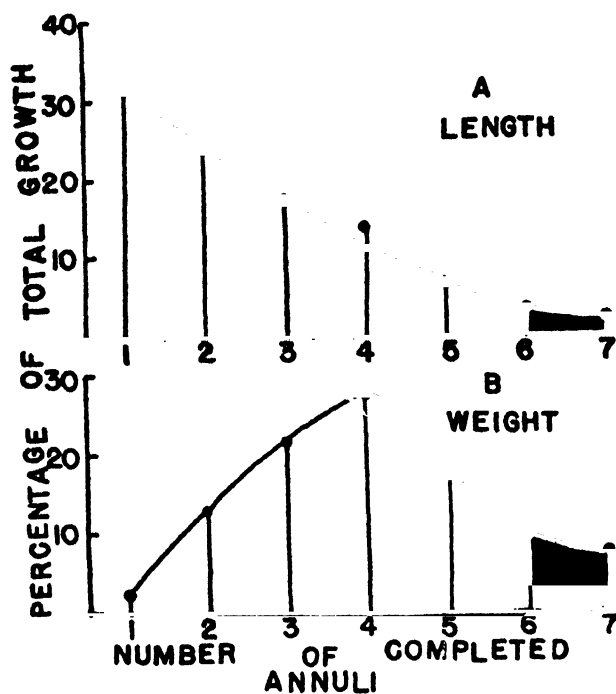
TEXT-FIG. 12.

Percentage of growth in individual year of life of the total growth in seven years plotted against the number of completed annuli.
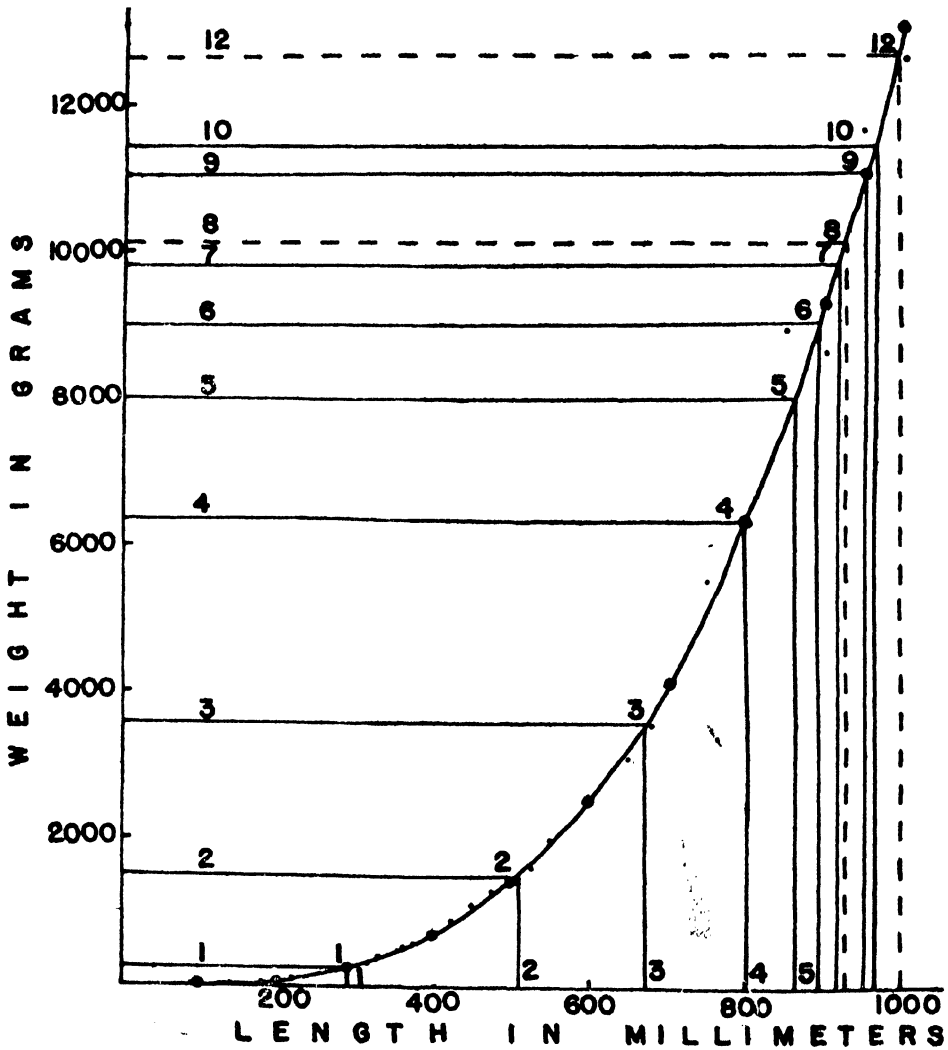
TEXT-FIG. 13.

The length-weight relationship curve of *Mrigal* with sizes and weights of *Mrigal* attained in different years of life.   Large black dots represent the calculated weights an (smaller ones show averages of a few observed weights of the corresponding lengths.

4

the length-weight relationship of *Mrigal* can be described by the following formulae :

Log Weight : —5.54534+3.221 Log Length.

The length-weight relationship worked out by the author (Jhingran, 1952) earlier was in respect of fork length as against total length dealt with here. Figure 13 shows the total-length-weight relationship curve on arithmetic grid which also shows the sizes and weights of *Mrigal* in millimetres and grams respectively reached in the first twelve years of life (data shown in Table 3).

RELATIONSHIP BETWEEN LENGTH AND GIRTH

With the practical object of deciding upon the minimum mesh limit, should the conservation of *Mrigal* fishery appear to make it necessary, a general length-girth relationship was studied. The limitations of this study are stated earlier under "Material and methods". 1,102 observations on fish ranging in total length from 200.0 mm. to 1,010 mm. and in girth from 82.6 mm. to 736 mm. have been utilised for his exposition. Figure 14 shows a plot of girth against total length
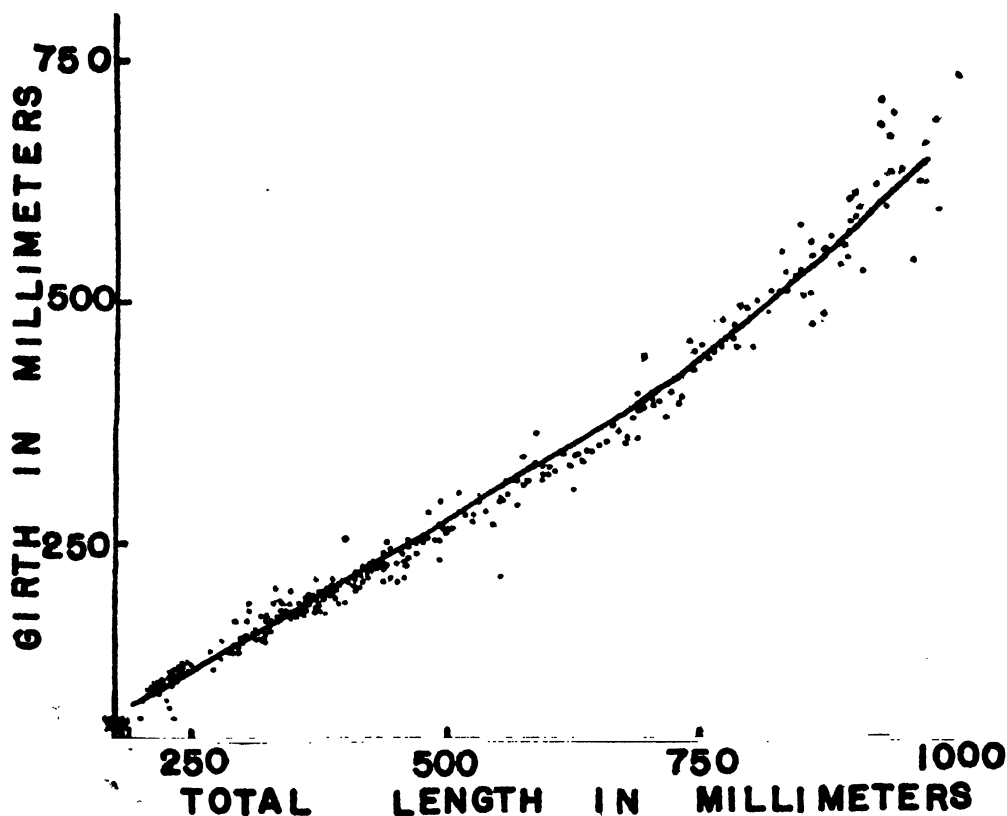
TEXT-FIG. 14.

Girth of *Mrigal* in millimetres plotted against total length in millimetres.

both in millimeters. The scatter diagram reveals a slightly curvilinear relationship. A plot on a double log scale renders a rectilinear form of scatter which is

shown in Figure 15. The length-girth relationship is described by the following formula :

$$\text{Log Girth} = -0.758780 + 1.1846 \text{ Log Length.}$$

LOG L = - 0.758780I + 1.1846 LOG G
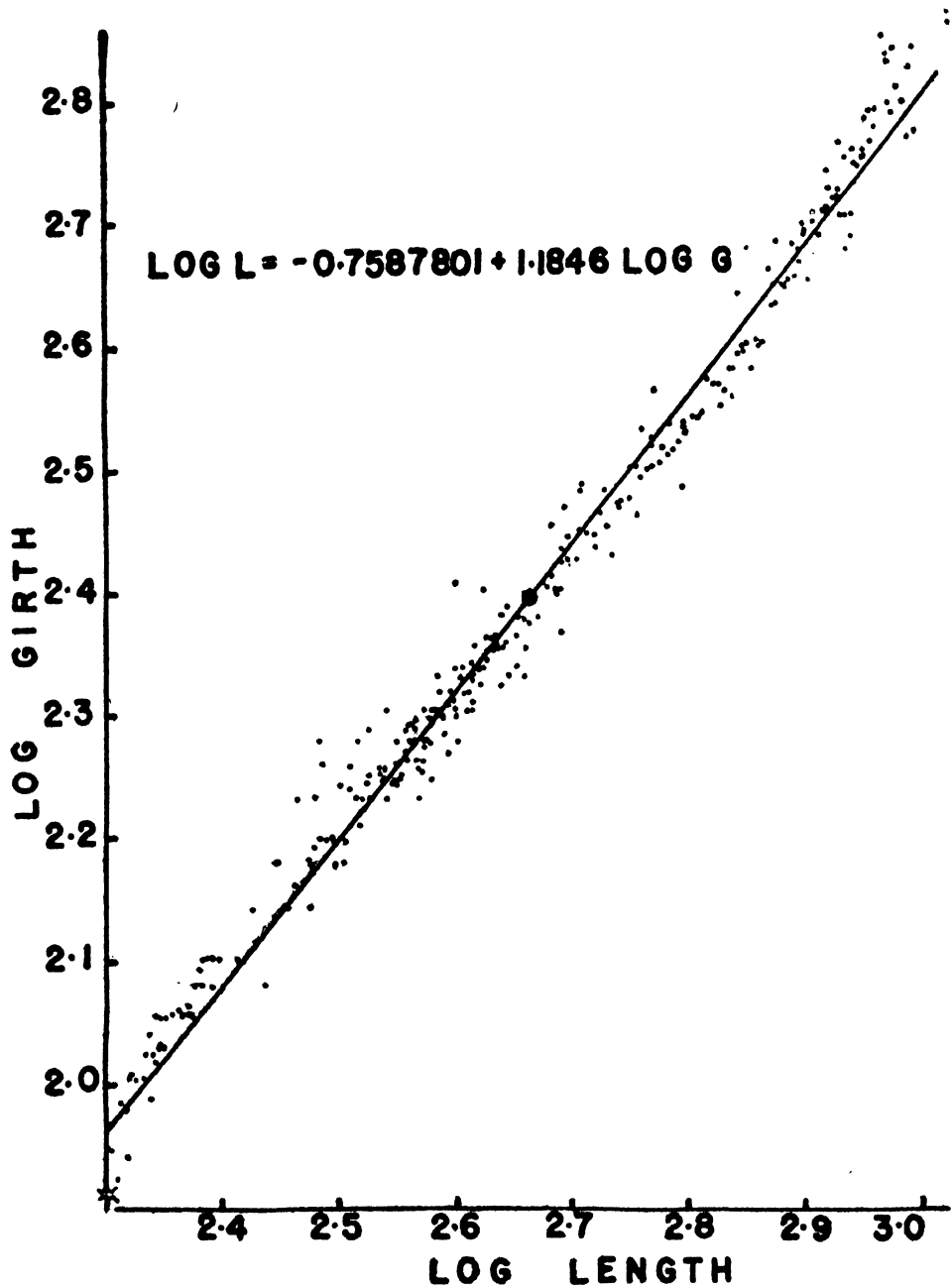
TEXT-FIG. 15.

Log girth of *Mrigal* plotted against log total length and the regression line to fit the scatter of observed points.

DISCUSSION

A systematic and thorough study of growth of *Mrigal* from any habitat has not yet been made in India. The fish is reported to grow 7 inches in $3\frac{1}{2}$ months (Basu, 1946) thus averaging 2 inches per month. Chacko and Ganapati (1951) report an attainment of 18″ to 24″ and a weight of 2.5 to 4.0 lbs. in one year in some tanks and swamps of the Godavari and Krishna districts. In Chetput Fish Farm, the same authors report that *Mrigal* has attained 22″–26″ and 3–5 lbs. in one year. They state that in Ichapur Fish Farm in N. Vizagapatam the fish attained a length of 12″ to 15″ by June in 1950. The growth rate is reported to be $1\frac{1}{2}$ to 2 inches per month in the first year in fertilised waters. In the present work month to month growth of *Mrigal* in River Ganga at Buxar has been traced upto third year-group state (Figure 7) and average sizes attained by the fish upto twelve years of life have been delineated by utilising the scale method. Some of the above stated records of growth by Chacko and Ganapati are phenomenal and probably demonstrate the difference between conditions under capture and culture fisheries. *Mrigal* in the Ganga at Buxar grows to an average size of 11.5″ 20.1″, and 26.4″ in the first three years and attains an average weight of 0.54, 3.33 and 7.98 lbs. respectively. The overall growth rate in length in the first three years is 32.3, 18.4 and 13.3 mm. per month and in weight 27.3, 105.5 and 175.5 gm. per month respectively.

A detailed analysis as to the size and age composition of the commercial catches, statistics of production, total fishing effort expended and catches in relation to appropriately devised unit of effort of the population involved, ought to be utilized to judge the path *Mrigal* fishery has been following during the course of its exploitation from ancient times to the present day. Such data, if available, would enlighten one as to the scope for more intensive exploitation or need for conservation. Depletion, if it occurs, is usually accompanied by certain symptoms, one of which is reduction in the average size and age of the fish available to the commercial gear as compared with those available in the earlier years of exploitation. At the present moment little is known about the spatial distribution or the geographical range of the population or populations involved, the morphological, anatomical or physiological characters with which to identify them, if more than one populations exist, and no catch statistics has been maintained by any agency in the past except for certain selected centres where the Allahabad Sub-Station of the Central Inland Fisheries Research Station has been making observations on catches for the last 5 or 6 years (Jhingran, 1956). However, efforts are now afoot to systematically start such studies over the entire River Ganga as would lead to the elucidation of the salient features of the population dynamics of the fisheries concerned. Determination of the age of fish being an essential prerequisite for the study of population dynamics of *Mrigal*, it is believed that the findings, reported here, open a new arena of investigation, which was hitherto closed, and it is hoped that future studies, conducted along proper lines, will lead to the formulation of a sound management policy of the fishery of this one of the most important Indian fresh water fish. It is desirable, however, to specifically state that utilization of scales to decipher age and growth is full of pitfalls. The line of approach pursued in the present study is indirect, though elaborate, and final confirmation of the issue will be got when either the "known age method" (Van Oosten, 1923 ; Applegate, 1947) or marking experiments tried. Elucidation of growth structure and developmental physiology with proper staining technique to demarcate areas of fast and slow growth on the scale surfaces, such as were worked out for the first time by Wallin (1957) on roach scales, if attempted on Indian species, will further enlighten the subject.

Studies on the growth rate and relationship between fish length and girth of *Mrigal*, presented in this paper, have a direct bearing on fixing minimum legal

size limit and mesh regulation, should the adoption of these common conservation measures be found necessary by the fishing industry to reap optimum catches and regulate the fishery. Although low productivity and colossal fishing mortality of the juveniles of *Mrigal* and other major carps (Jhingran, 1956 and 1957 : Jhingran and Chakraborty, 1958) are to be treated as warning signals (Russel, 1942) no final verdict on the issue of depletion of the fishery is warranted at the present state of knowledge. Demonstration of depletion is essentially a time-consuming process, and sustained studies, along lines indicated here, will lead to a better understanding of the fishery on the basis of which its conservation has to be planned.

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REFERENCES

Alvord, William (1953). Validity of age determinations from scales of brown trout, Rainbow trout, and brook trout. *Trans. Amer. Fish. Soc.*, **83**, 91–103.

Applegate, Vernon C. (1943). Partial analysis of growth in a population of mudminnows *Umbra limi* (Kirtland). *Copeia*, **2**, 92–96.

———— (1947). Growth of some lake trout, *Cristivomer namaycush* of known age in inland Michigan lakes. *Ibid.*, 4237–241.

Basu, S. P. (1946). Proceedings of the conference of fishery officers of the Government of Bengal held on 6, 7 and 8 November, 1945. Appendix D.

Baudelot, Emile (1873). Recherches sur la structure et le développment des ecailles des poissons osseux *Arch. Zool. exp. gen.*, **2**, 87–244, 427–480.

Beckman, Wm. C. (1941). Increased growth rate of rock bass, *Ambloplites rupestris* (Rafinesque), following reduction in the density of the population. *Trans. Amer. Fish. Soc.*, **70**, 143–148.

———— (1942). Length-weight relationship, age, sex ratio, and food habits of the smelt (*Osmerus mordax*) from Crystal Lake, Benzie Country, Michigan. *Copeia*, **2**, 120–124.

———— (1943a). Annulus formation on the scales of certain Michigan game fishes. *Pap. Mich. Acad. Sci.*, **28** 281–312.

———— (1943b). Further studies on the increased growth rate of the rock bass, *Ambloplites rupestris* (Rafinesque) following the reduction in density of the population. *Trans. Amer. Fish. Soc.*, **72**, 72–78.

———— (1949). The rate of growth and sex ratio of seven Michigan fishes. *Ibid.*, **76**, 63–81.

Bukht (1940). Bulletin No. 1 Co-operative Fishery, Calcutta.

Carlander, Kenneth D. (1945a). Age, growth, sexual maturity and population fluctuations of the yellow pike-perch, *Stizostedion vitreum vitreum* (Mitchill) with reference to the commerical fisheries, Lake of Woods, Minnesota. *Trans. Amer. Fish Soc.*, **73**, 90–107.

———— (1945b). Growth length-weight relationship and population fluctuations of the tullibee, *Leucichthys artidi tullibee* (Richardson), with reference to the commerical fisheries, Lake of the Woods, Minnesota. *Ibid.*, **73**, 125–136.

———— (1948). Growth of yellow pikeperch, *Stizestedion vitreum vitreum* (Mitchill) in some Iowa lakes, with a summary of growth rates reported in other areas. *Iowa St. Coll. J. Sci.*, **22** (3), 227–237.

———— (1950a). Growth rate studies of saugers, *Stizostedion canadese canadese* (Smith) and yellow perch, *Perca flavescens* (Mitchill), from lake of the Woods, Minnesota. *Trans. Amer. Fish. Soc.*, **79**, 30–42.

———— (1950b). Some considerations in the use of growth data derived from scale studies *Ibid.*, **79**, 187–194.

———— (1956). Appraisal of methods of fish population study—Part I. Fish growth rate studies : Techniques and role in surveys and management. *Trans. Twenty-first N. Amer. Wildlife Conf.* March 5, 6 and 7, 1956. Washington.

Chacko, P. I. and Dixitulu, J. V. H. (1951). Further observations on the radii of scales of *Hilsa ilisha* (Ham.). *Proc. 38th Indian Sci. Cong.*, Bangalore. Abstracts Parts III, p. 227.

Chacko, P. I. and Ganapati, S. V. (1951). Bionomics of the Mrigal, *Cirrhina mrigala* (Ham.) in South Indian waters. *J. Bombay nat. Hist. Soc.*, **50**, (1), 13–19.

Chacko, P. I. and Krishnamurty, B. (1950). A biometrical study of the *Hilsa ilisha* (Ham.) in he Godavary river. *J. Bomb. nat. Hist. Soc.*, **49**, (2), 315–316.

Chacko, P. I., Zobairi, A. R. K. and Krishnamurty, B. (1948). The radii of scales of *Hilsa ilisha* (Hamilton) as an index of growth and age. *Curr. Sci.*, **5**, 158–159.

Chevey, P. (1930). C. Essai d' application de la method de lecture des ecailles a P etude do la croissance de poissons du Grand Lac du Cambodge at du Tonle-Sap. *C. R. Acad. Sci., Paris*, **191**, 1475–1477.

————— (1932). Sur la nature de l'influence exercie par la foret inondee de Grand Lac du Cambodge sur la vitesse de croissance des poissons. *Ibid.*, **195**, 1108–1110.

Chidambaram, K. (1950). Studies on length-frequency of oil sardine, *Sardinella longiceps* Cuv. and Val. and on certain factors influencing their appearance on the Calicut coast of Madras Presidency. *Proc. Indian Acad. Sci.*, **31**, 252–286.

Chidambaram, K. and Krishnamurty, C. G. (1951). Growth rings on the otoliths of the Indian mackerel, *Rastrelliger kanagurta* Russel. *Proc. 38th Indian Sci. Congr.*, Bangalore Abs . Part III, p. 223.

Creaser, Charles W. (1926). The structure and growth of scales of fishes in relation to the interpretation of their life-hostory, with special reference to the sunfish, *Eupmotis gibbosus*. Univ. Mich. Mus. of Zool. Misc. Pub. No. 17, pp. 1–82, Ann Arbor.

Devanesan, D. W. (1943). A brief investigation into the causes of the fluctuations of the annual fishery of the oil sardine of Malabar *Sardinella longiceps* Cuv. and Val. Determination of its age and an account of the discovery of its eggs and spawning grounds Rept. No. 1, *Madras Fish. Bull.*, **28**, 1–38.

Devasundaram, Peter, M. (1952). Scale study of *Mugil cephalus* Linnaeus of Chilka Lake. *J. Madras Univ.*, B **22**, (1), 147–163.

Eschemeyer, Paul (1950). The life history of the walleye in Michigan. *Bull. Inst. Fish. Res.* Michigan Dept. Conservation No. 3, 99.

Foerster, R. E. (1936). An investigation of the life history and propagation of the Sokeye salmon (*Oncorhynchus nerka*) at Cultus Lake, British Columbia. No. 5. The life history cycle of the 1926 year class with artificial propagation involving the liberation of the free swimming fry. *J. Biol.*, Canada, **2**, (3), 311–333.

Godfrey, H. (1955). On the Ecology of the Skeena River Whitefishes, *Coregonus* and *Prosopium*. *J. Fish. Res. Bd Can.*, **12**, (4), 499–542.

Graham, Michael (1929). Studies on Age determination in Fish, Part II. A Survey of the literature. Ministry of Agriculturac and fish, Fish Investigation Series No. 2, pp. 1–50.

Hile, Ralph (1931). Rate of growth of fishes of Indiana Invest. of Indiana. Lakes No. II Dept. Conserv. Div. Fish and Game, Indiana Publ. No. 107, pp. 9–55.

————— (1936). Age and Growth of the cisco, *Leucichthyas artedi* (Le Sueur), in the lakes of the northeastern high lands Wisconsin. *Bull. U. S. Bur. Fish.*, **48**. No. 19, 211–317.

————— (1941). Age and growth of the rock bass, *Ambloplites rupestris* (Rifinesque) in Nebish Lake, Wisconsin. *Trans. Wis. Acad. Sci. Arts and Lett.*, **33**, 189–337.

————— (1942). Growth of the rock bass, *Ambloplites rupestris* (Rafinesque), in five lakes of Northeastern Wisconsin. *Trans. Amer. Fish. Soc.*, **71**, 131–143.

Hora, S. L. and Nair, K. K. (1940). Further observations on the bionomics and fishery of the Indian shad *Hilsa Ilisha* (Hamilton) in Bengal waters. *Rec. Indian Mus.*, **42**, (1), 35–50.

Hornell, J. and Naidu, M. R. (1924). A contribution to the life history of the Indian sardine with notes on the plankton of the Malabar coast. *Madras Fish. Bull.*, **17**, Rept. 5, 129–197.

Hutton, J. Arthur, (1921). The Literature on fish Scales. *Salmon and Trout Maga.*, **26**, 203–217.

Jhingran, V. G. (1952). General length-weight relationship of three major carps of India. *Proc. nat. Inst. Sci. India*, **18**, (5), 449–460.

————— (1956). The capture fishery of River Ganga at Buxar (Bihar, India) in the years 1952–1954. *Indian J. Fish.*, **3**, (1), 197–215.

————— (1957). Age determination of the Indian major carp *Cirrhina mrigala* (Ham.) by means of scales. *Nature*, **179**, 468–469.

————— and Chakraborti, R. D. (1958). Destruction of major carp fingerlings in a section of River Ganga and its probable adverse effects on fish production. *Indian J. Fish.* **5**(2), 291–299.

Jobes, F. W. (1933). Preliminary report on the age and growth of the yellow perch from Lake Erie as determined from a study of its scales. *Pap. Mich. Sci.* **16**, 643–652.

————— (1943). The age, growth and bathymetric distribution of Reighard's chub *Leucichthys reighardi* Keolz, in Lake Michigan. *Trans. Amer. Fish. Soc.*, **72**, 108–135.

————— (1949a). The age, growth and bathymetric distribution of the bloater *Leucichthys Hoyi* (Gill) in Lake Michigan. *Pap. Mich. Acad. Sci.* **33**, 135—172.

————— (1949 b). The age, growth and distribution of the long jaw cisco, *Leucichthys alpenae* (Koelz), in Lake Michigan. *Trans. Amer. Fish. Soc.*, **76**, 215–247.

Jones, S. and Menon, P. M. G. (1951). Observations on the life history of the Indian shad, *Hilsa ilisha* (Hamilton). *Proc. Indian Acad. Sci.*, **33**, (3), 101–125.

Kennedy, W. A. (1954 a). Tagging returns, age studies and fluctuations in abundance of Lake Winnipeg Whitefish, 1931–1951. *J. Fish Res. Bd Can.*, **11**, (3), 284–309.

———— (1954b). Growth, maturity and mortality in the relatively unexploited Lake Trout, *Critivomer namaycush* of Great Slave Lake. *Ibid.*,**11**, (6), 827–852.

Menon, D. M. (1953). The determination of age and growth of fishes of tropical and Sub-tropical waters. *J. Bombay nat. Hist. Soc.*, **51**, (3), 623–635.

Mohr, Erna. W. (1927). Bibliographic der Altersund Wachsumsbestimmung bei Fischen. *J. Con. int. Exlor. Mer.*, No. 2 (2), p. 236–258.

———— (1930). *Idem.*, Nachtrage und Fortsetzung. *Ibid*, No. 5 (1) pp. 88–100.

———— (1934). Idem., Nachtrage und Fortsetzung. *Ibid.* No. (3) pp. 377–391.

Nair, R. V. (1949). The growth rings on the otoliths of the oil sardine *Sardinella longiceps* Cuv. and Val. *Curr. Sci.*, **18**, 9–11.

Pantulu, V. R. (1956) Studies on the biology of the Indian freshwater eel, *Anguilla bengalensis* Gray. *Proc. nat. Inst. Sci. India*, **22**, B, (5), 259–280.

Parsons, John W. (1953). Growth and habits of the redeye bass. *Trans. Amer. Fish. Soc.*, **83**, 202–211.

Pillay, T. V. R. (1954). The biology of the Grey mullet, *Mugil tade* Forskal with notes on its fishery in Bengal. *Proc. nat. Inst. Sci. India*, **20**, (2), 187–217.

Raj, B. Sundara. (1951). Are scales an index of age and growth of Hilsa ? *Ibid.*, **17**, (1), 1–6.

Rao, S. Ranga. (1934). A statistical study of the growth in *Therapon jarbua* Day. *Proc. 21st. Indian Sci. Congr.* Bombay, Abstracts Pt. III. p. 249.

———— (1935). A study of the otoliths of *Psettodes erumei* (Bl. Schn.) *Proc. 22nd. Indian Sci. Congr.* Calcutta, Abstracts Pt. III p. 319.

Ricker, W. E. (1942). The rate of growth of bluegill sunfish in lakes of Northern Indiana. *Invest. Indiana Lakes and Streams.* **2** pp. 161–214.

Russel, E. S. (1942). The Overfishing Problem. Cambridge Univ. Press.

Sarojini, K. K. (1957). Biology and fisheries of the grey mullets of Bengal. I. Biology of *Mugil parsia* Hamilton with notes on its fishery in Bengal. *Indian J. Fish.*, **4**, (1), 160–207.

Seshappa, G. and Bhimachar, B. S. (1951). Age determination studies in fishes by means of scales with special reference to the Malabar sole. *Curr. Sci.* **20**,, 260–262.

———— (1954). Studies on the age and growth of the Malabar sole, *Cynoglossus semifasciatus* Day. *Indian J. Fish.*, **1**, 145–162.

Smith, S. B. (1955). The relation between scale diameter and body length of Kamloops trout, *Salmo gairdneri kamloops*. *J. Fish. Res. Bd Can.*, **12**, (5), 742–753.

Sprugel, George (Jr.) (1953). Growth of bluegill in a new lake with particular reference to false annuli. *Trans. Amer. Fish. Soc.*, **83**, 58–75.

———— (1955). The growth of green sun fish *Lepomis cyanellus* in Wall Lake. *Iowa St. Coll. J. Sci.*, **29**, (4), 707–719.

Stroud, Richard H. (1948). Growth of the basses and black crappie in *Norris* Reservoir, Tennessee. *J. Tenn. Acad. Sci.*, **23**, (1), 31–99.

———— (1949 a). Growth of Norris Reservoir walleye during the first twelve years of impoundment. *Wildlife Mgmt*, **13**, (2), 157–177.

———— (1949 b). Rate of growth and condition of game and pan fish in Cherokee and Douglas Reservoirs, Tennessee and Hiwassee Reservoir. North Corolina. *Tenn. Acad. Sci.*, **24**, (1). 60–74.

Taylor, Hardon F. (1916). The structure and growth of the scales of squteaque and the pigfish as indicative of life history. *Bull. U. S. Bur. Fish.*, **24**, No. 823, 285–330, Washington.

Thompson, J. Stuart (1904). The periodic growth of scales in Gadidae as an index of age. *J. Mar. Biol. Assoc.* New Series 7, No. 1, 1–109, Plymouth.

Van Oosten, John (1923). The whitefishes (*Coregonus clupeaformis*) : A study of the scales of whitefishes of known ages. *Zoologica*, (N. Y.) **2**, 381–412.

———— (1929). Life history of the lake herring *Leucichthys argedi* (Lesueur) of Lake Huron as revealed by its scales, with a critique of the scale method. *Bull. U. S. Bur. Fish.*, **44** No. 1053, 265–428, Washington.

———— (1937). The Age, growth and sex ration of the Lake Superior longjaw, *Leucichtvs zenithicius* (Jordon and Evermann). *Pap. Mich. Acad. Sci.*, **22**, 691–711.

———— (1938). The age and growth of the lake Erie sheephead, *Aplodinotus grunniens* Rafinesque. *Ibid.*, **23**, 651–658.

———— (1939). The age, growth, sexual maturity, and sex ration of the common whitefish, *Coregonus clupeaformis* (Mitchill) of Lake Huron. *Ibid.*, **24**, 194–221.

———— (1941). The age and growth of freshwater fishes. A Symposium on Hydrobiology Univ. of Wisconsin Press. pp. 196–205.

———— (1942). The age and growth of lake Erie white bass, *Lepibema chrysops* (Rafinesque). *Pap. Mich. Acad. Sci.*, **27**, 307–334.

Wallin, Olle. (1957). On the growth structure and development physiology of the scales of fishes. Inst. Freshw. Res. Drottningohm. Rept. No. 38, 385–447.

THE UTILIZATION OF MONOSACCHARIDES BY *PESTALOTIA BANKSIANA* AND *P. CITRI*

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ABSTRACT

The utilization of eight monosaccharides by *Pestalotia banksiana* and *P. citri* was studied in detail. The influence of three different types of nitrogen (viz. ammonium, organic and nitrate) on the assimilation rate of different sugars was also investigated. Circular paper chromatographic method was employed for daily analysis of the medium.

Glucose was the best monosaccharide for the present organisms. Maximum dry weight was attained when this sugar was used in combination with ammonium chloride.

Mannose, galactose, arabinose and xylose were also satisfactory sources. Levulose was good for the growth of *P. banksiana* only.

Rhamnose and sorbose were very poor sources. Chromatographic analysis of the medium showed that these sugars were not consumed completely by any of the organisms even in 15 days.

Addition of sorbose had very pronounced inhibitory effect on other monosaccharides. Utilization of all other sugars was delayed in presence of sorbose.

Ammonium nitrogen was superior to organic or nitrate nitrogen.

Generally all the polysaccharides or oligosaccharides first get hydrolysed into simple soluble components before they can be utilized as food by the cells of the micro-organisms. The action of proper extracellular enzyme is necessary to bring about the reaction required to change the substance into more simple soluble compounds which can then diffuse through the cell membranes. Recent investigations of Tandon and Bilgrami (1958) have shown that two species of *Pestalotia* viz. *P. banksiana* and *P. citri* utilized some of the common oligosaccharides like raffinose, sucrose and maltose etc., after hydrolysis. As the monosaccharides were the ultimate sugars to be produced in the medium, it was decided to study the influence of 8 different monosaccharides on the rate and amount of growth of these two fungi.

Previous observations of the authors (Tandon and Bilgrami, 1957, 1958) had shown that the utilization of sugars by the species of *Pestalotia* was also dependent on the type of nitrogen source present in the substratum. In view of these results it was decided to supply three different types of nitrogen sources individually in combination with each of the monosaccharide used in the present investigation.

Influence of mixing sorbose (which has generally been reported to be very poor carbon source for most of the fungi) with other sources of carbon was also studied.

MATERIALS AND METHODS

Single spore cultures of *Pestalotia banksiana* and *P. citri* used in the earlier investigations were employed. Eight monosaccharides (viz., glucose, levulose, galactose, mannose, sorbose, arabinose, xylose and rhamnose) and three nitrogen sources viz., ammonium chloride, asparagin and potassium nitrate, were individually supplied to the culture medium*. It had 4000 mgs. of carbon and 490 mgs. of nitrogen per litre. Purest available chemicals of E. Merck or B.D.H. make were used. 25 ml. of liquid nutrient (contained in 150 ml. Erlenmeyer flasks)

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*$KH_2 PO_4$—1·75 gms, $MgSO_4.7H_2O$. 0·75 gm and double distilled water 1 litre.

was used to culture the organisms. The various media were autoclaved at 15 lbs. pressure for 15 mts. Both the fungi were daily inoculated at a fixed time ($\pm$ 25 mts) for 15 days in the autoclaved solutions.

Triplicate sets were used in every case. Fungal colonies from each set were separately filtered on the 16th day. Filtrate of each day was chromatographically analysed while dried fungal mycelium was used as a measure of growth. Circular paper chromatograms were used. Butanol-acetic acid-water (4 : 1 : 5) and aniline diphenyl amine phosphate served as solvent and spray reagent respectively. The average Rf values of various sugars were measured and have been recorded in the text.

TABLE I

Showing the dry weights (in mgs) of Pestalotia banksiana and P. citri obtained on 6th, 11th and 16th day on different combinations of monosaccharides and three sources of nitrogen.

Monosaccharides	Days of incubation	P. banksiana			P. citri		
		Nitrogen sources			Nitrogen sources		
		Amm. chloride	Asparagin	Potassium nitrate	Amm. chloride	Asparagin	Potassium nitrate
Glucose	5	40	35	32	31	27	26
	10	69	66	58	56	42	44
	15	96	84	76	69	59	58
Levulose	5	39	40	33	20	15	10
	10	75	61	57	31	27	18
	15	92	78	74	44	36	22
Mannose	5	30	32	21	26	20	21
	10	57	59	39	44	35	32
	15	76	70	50	54	42	38
Galactose	5	37	33	24	28	21	22
	10	66	58	43	41	39	37
	15	82	68	56	57	48	46
Sorbose	5	9	2	2	6	4	3
	10	16	11	6	18	13	5
	15	20	14	8	20	16	5
Arabinose	5	27	21	22	28	25	24
	10	44	36	32	46	44	37
	15	56	48	40	60	52	48
Xylose	5	33	29	25	18	15	13
	10	51	48	43	30	27	21
	15	68	60	54	40	33	28
Rhamnose	5	21	9	8	10	9	8
	10	26	19	16	19	16	15
	15	32	25	23	26	21	19

EXPERIMENTAL

It is evident from Table No. 1 that glucose in combination with NH_4Cl served as best monosaccharide for both the species of *Pestalotia*. *P. banksiana* exhibited almost similar growth on levulose also, while *P. citri* showed much less growth on this substance than on glucose. Mannose and galactose were also satisfactory

sources but they were decidedly inferior to glucose. Sorbose was the poorest monosaccharide. Amongst the pentoses arabinose was slightly superior to galactose or mannose for *P. citri*, while *P. banksiana* exhibited greater liking for xylose than for arabinose. Rhamnose was a poor source but it was better than sorbose for both the species of *Pestalotia*.

The influence of addition of sorbose on the utilization of other monosaccharides has been recorded in Table 2.

A comparison of Tables 1 and 2 shows that addition of sorbose to glucose, levulose, mannose or galactose or to any of the pentoses resulted, in limiting the efficiency of these sugars also. It was observed that the vegetative growth of both the organisms on different sugars was reduced to less than half by the addition of sorbose.

TABLE II

Showing the influence of addition of Sorbose on the dry weight yield (in mgs.) of P. banksiana *and* P. citri.

Monosaccharides	No. of Days of incubation	P. banksiana			P. citri		
		Nitrogen sources			Nitrogen sources		
		NH₄Cl	Asparagin	Potassium nitrate	Amm. chloride	Asparagin	Potassium nitrate
Glucose	5	15	11	10	12	9	10
and	10	31	26	23	23	17	21
Sorbose	15	44	36	32	30	24	28
Levulose	5	14	10	8	11	8	9
and	10	32	23	21	19	18	16
Sorbose	15	45	34	30	28	24	22
Mannose	5	11	10	7	8	6	6
and	10	23	21	15	17	14	11
Sorbose	15	33	32	22	26	21	16
Galactose	5	13	9	8	5	5	6
and	10	24	20	17	11	12	10
Sorbose	15	34	28	26	18	14	13
Arabinose	5	8	7	5	9	8	6
and	10	18	16	12	20	15	13
Sorbose	15	27	25	18	29	20	19
Xylose	5	12	9	7	7	6	5
and	10	25	19	14	12	11	8
Sorbose	15	31	24	19	17	14	10
Rhamnose	5	8	5	5	6	4	5
and	10	13	11	8	10	9	8
Sorbose	15	15	13	9	13	11	8

The results of chromatographic analysis of different media (using NH₄Cl as nitrogen source) are summarized in Table 3.

TABLE III

Showing the results of the Chromatographic analysis of different media during the growth of P. banksiana *and* P. citri

Name of the monosaccharide	P. banksiana		P. citri	
	A	B	A	B
Sorbose Rf 0.72	15	15	15	15
Glucose Rf 0.65	7	10	10	13
Mannose Rf 0.69	10	13	12	15
Levulose Rf 0.70	8	10	11	15
Galactose Rf 0.64	8	11	14	15
Arabinose Rf 0.71	11	14	9	13
Xylose Rf 0.74	12	15	13	15
Rhamnose Rf 0.82	15	15	15	15

(Column A denotes the number of days taken in utilizing a single monosaccharide. Column B indicates the number of days when sorbose was used in combination with a particular monosaccharide).

Table 3 clearly shows that utilization of all the monosaccharides by both the species of *Pestalotia* was delayed when sorbose was mixed with them. Rhamnose was not consumed either singly or in combination with sorbose up to 15 days.

DISCUSSION

Monosaccharides are the most easily assimilable carbohydrates by micro-organisms. Our recent investigations have revealed that the complex carbohydrates which were usually present in the host plant, are first converted into simpler mono-saccharides like glucose and levulose and then utilized. The results of the present investigation showed that glucose is the best monosaccharide. Several investigators have reported that this sugar is the most efficient source of carbon for a large number of fungi. Some have reported that its addition to other sugars had a stimulatory effect for some of the fungi investigated by them. Our earlier investigations had shown that maltose (a disaccharide, composed of two glucose units) was the best oligosaccharide for both *Pestalotia banksiana* and *P. citri*. A comparison with previous results (Tandon and Bilgrami 1958) shows that maltose is a much better source than glucose for both the species of *Pestalotia*. The superiority of maltose over glucose has also been reported by Brock (1951) and Agarwal (1955) for *Morchella esculenta* and *Curvularia penniseti* respectively. Our unpublished work showed that starch is also a comparatively better source than glucose. Blank and Talley (1941) also found starch and maltose to be better sources for *Phymato-trichum omnivorum*. They suggested that impurities of the chemicals might have been responsible for this behaviour. Maltose and starch of extra pure qualities were used by the authors and it is thus clear that the results are not due to any impurity. It appears that the behaviour of maltose or starch may be connected with the availability of *active glucose* during decomposition of those substances by *Pestalotia banksiana* and *P. citri*.

Chromatographic studies showed that with the exception of rhamnose and sorbose all other sugars are consumed by the present organisms within the incubation period. The results also showed that the addition of sorbose to the culture medium results in slow utilization of good sources also. Levulose, mannose, xylose and galactose, which were individually consumed by *P. citri* in 11, 12, 13 and 14 days respectively, were not consumed completely even in 15 days when used in association with sorbose. It was also clear that addition of sorbose to any of these

monosaccharides considerably reduces the dry weight. It was interesting to find that mannose lost its efficiency to such an extent that mycelial growth of *P. citri* on a mixture of mannose-sorbose medium was similar to that on sorbose alone. Lilly and Barnett (1953) have also reported the inhibitory effect of sorbose for a number of fungi. They reported that inhibitory action of sorbose is greater in presence of sucrose or maltose than with glucose. They further mentioned that in fact this sugar (sorbose) may be stimulatory in presence of glucose and inhibitory in presence of sucrose or maltose. The size and complexity of the sugar molecule has been suggested to be the possible reason for this behaviour. The present results, however, showed that addition of sorbose to other monosaccharides was not beneficial in any case. The reason for the toxic effect of sorbose on the growth of micro-organisms is not clear to any worker so far. Chromatographic analysis of the medium showed that sorbose was not utilized by any of the two species of *Pestalotia* up to 20 days though the autolysis of the fungal mycelium had already started by that time.

Lilly and Barnett (1953) recorded interesting results of the nitrogen source on the rapidity with which sorbose inhibition was overcome. They reported that hydrolysed casein counter acted sorbose inhibition more than asparagin or nitrate nitrogen. The results reported herein showed that ammonium nitrogen was most suitable source of nitrogen. It is felt that superiority of ammonium chloride is due to the specific choice of these fungi for the ammonium nitrogen and not on account of the fact that ammonium chloride prevents the inhibitory effect of sorbose. This statement is supported by the fact that these fungi as well as several other species of *Pestalotia* have been reported to exhibit greater choice for ammonium nitrogen than for nitrate or organic nitrogen.

Amongst pentoses rhamnose was inferior to arabinose or xylose. Rhamnose is a methyl pentose i.e. it has CH_3 grouping in its structure, while the other two pentoses do not have this group. It is possible that CH_3 group in rhamnose may have some prominent effect in the type of growth observed on this substance.

REFERENCES

Agarwal, G. P. (1955). Cultural and pathological studies of fungi imperfectii. D.Phil. Thesis, University of Allahabad.

Blank, L. M., and Talley, P. J. (1941). The carbon and carbohydrate activity of *Phymatotrichum omnivorum. Amer. J. Bot.*, **28**, 564–569.

Brock, T. D. (1951). Studies on nutrition of *Morchella esculenta. Mycologia*, **43**, 402–422.

Lilly, V. G. and Barnett, H. L. (1953). The utilization of sugars by fungi. *Bull W. Va agric. Exp. Sta.*, 362T.

Tandon, R. N. and Bilgrami, K. S. (1958). The utilization and synthesis of oligosaccharides by two species of *Pestalotia. Proc. nat. Inst. Sci. India.*, **24**, 118-124.

CONTRIBUTIONS TO THE EMBRYOLOGY OF PALMAE

I. SABALEAE

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Abstract

The embryology of the following eleven species of the tribe Sabaleae is described : *Licuala grandis* H. Wendel, *L. spinosa* Wurumb., *L. peltata* Roxb., *Trachycarpus* sp. H. Wendel., *Livistona rotundifolia* Mart., *L. chinensis* R. Br., *Pritchardia pacifica* Seem. et Wendel., *Washingtonia* sp., *Sabal adansoni* Guers., *S. blackburniana* Glazebr., and *S. palmetto* Lodd.

In all species, the flowers are regular, trimerous and hermaphrodite. The floral organs arise in acropetal sequence.

The anther wall consists of a heavily cutinised epidermis, a fibrous endothecium, 2 median layers and a secretory tapetum of binucleate cells. In *Traychycarpus* the tapetal cells store starch and in *Sabal palmetto* their inner walls become cutinised. There is a secondary increase of sporogenous cells. Cytokinesis of the p.m.c. is due to simultaneous cell plate formation. Microspore tetrads are either tetrahedral or bilateral. Mature pollen grains are 2-celled, ellipsoidal, monocolpate and aporate. The generative cell is crescent-shaped with tapering ends and the vegetative cytoplasm is packed with starch.

The ovules are erect, bitegmic and crassinucellate; they are hemi-anatropous in *Livistona rotundifolia* and *Sabal* sp., but anatropous in other species. Two or three vascular bundles enter the funicle and branch slightly in the chalaza. A well developed funicular obturator is present in *Livistona* and *Sabal* sp. An endothelium is present in *Pritchardia, Washingtonia, Trachycarpus* and *Licuala*. The micropyle is formed only by the inner integument in *Sabal* and *Washingtonia*, but by both the integuments in other genera. A nucellar cap is formed in *L. rotundifolia*. A prominent development of chalaza is characteristic of all species.

The archesporium of the ovule is 1-celled; it cuts off the primary parietal cell. Megaspore tetrads are linear, T-shaped or ⌐-shaped. The embryo sac develops according to the *Normal*-type. The synergids show lateral protuberances. The polar nuclei fuse before fertilization. The antipodals, which are large in *Pritchardia*, persist till a few endosperm nuclei are formed.

Fertilization is porogamous; the passage of pollen tubes is facilitated by the transmitting tissue of the style and funicular obturator where present. The endosperm is of the nuclear type and becomes cellular by simultaneous cell plate formation. The endosperm cells are disposed in regular radial series; they are thick walled due to storage of hemi-cellulose. During seed development the chalaza grows and forms a columnar structure in *L. rotundifolia;* in *Licuala grandis* the chalaza develops warty protuberances which make the endosperm ruminate.

Only the terminal cell of the 2-celled proembryo forms the embryonal mass; the basal cell forms the suspensor. The mature embryo shows a massive lateral cotyledon which envelops the primary axis. The cells of the cotyledon are rich in reserve food materials. The plumule shows several primordia of scale and vegetative leaves. During germination of seed the cotyledon forms a short or long tube in which the primary axis is enclosed.

Introduction

The Palmae is a large family with 210 genera and 4,000 or more species most of which are distributed in the tropics of both hemispheres. There is considerable diversity of opinion regarding the taxonomic position and affinities of the family. Rendle (1953) includes it in the order Spadiciflorae along with Lemnaceae, Araceae, and Cyclanthaceae. The reasons for such a grouping are : "the relative size of the embryo and endosperm, the presence of a spathe and the association of a great number of small inconspicuous flowers in often huge inflorescences". Hutchinson (1934), however, feels that these characters are of little taxonomic importance and that the palms and aroids have nothing in common. He separates them

widely and places the palms by themselves in the order Palmales. The Araceae and Lemnaceae are included in the order Arales and Cyclanthaceae in the Cyclanthales of the second division of the monocotyledons, the Corolliferae. Hutchinson follows the classification of Engler and Prantl (1895) with this difference that he treats their series (Principes, Spathiflorae and Synanthae) as orders. Bentham and Hooker (1862–83), on the other hand, include Palmae along with Flagelleriaceae and Juncaceae in the series Calycinae and place Cyclanthaceae, Araceae and Lemnaceae in the series Nudiflorae. Lawrence (1955) feels that there is much need for taxonomic and morphological studies in the family.

PREVIOUS WORK

There are several works on the taxonomy and morphology of the palms: Drude (1895 in Engler and Prantl), Bailey (1930–1949), Dahlgren (1936), Martius (1823–1850), Blatter (1926), etc. though there are no recent monographs. The embryological studies are meagre and sometimes the reports of the investigators are mutually contradictory. Schnarf (1931) who reviewed the previous work, recommended a reinvestigation for amplification and confirmation of the data. Very little work has been done since publication of Schnarf's book. Juliano (1931) studied the morphology of the male flower of *Cocos nucifera*. Swamy (1942) has written a short note on the embryo sac development of *Areca catechu*. The report of De Poerck (1950) that the embryo sac in *Elaeis guinensis* develops according to the *Adoxa*-type has been contradicted by Kajale and Ranade (1953) who found that it develops according to the normal type. Bosch (1947) studied the cytology and floral anatomy of some members. Selvaratnam (1952) described the mature embryo of the areca nut and compared it with that of the Gramineae. The present writer has described the development of the embryo in *Areca catechu* (C. V. Rao, 1955).

MATERIALS AND METHODS

This article embodies the writer's observations on the organogeny, structure of the inflorescence and flower, development and structure of the anther and ovule, male and female gametophytes, endosperm and seed, fruit and germination of seed in the following members of the tribe Sabaleae : *Licuala grandis* H. Wendel., *L. spinosa* Wurumb., *L. peltata* Roxb., *Trachycarpus* sp., *Livistona rotundifolia* Mart., *L. chinensis* B. Br., *Pritchardia pacifica* Seem et Wendel., *Washingtonia* sp., *Sabal adansoni* Guers., *S. blackburniana* Glazebr., and *S. palmetto* Lodd.

All species studied are cultivated ornamental plants with flabelliform leaves. The material of *Licuala grandis*, *Livistona rotundifolia* and *Pritchardia* was collected from the local municipal park; that of *Sabal palmetto* and *Washingtonia sp.* was collected from Bobbili, Srikakulam District. Fixed materials of *Licuala spinosa*, *L. peltata*, *Trachycarpus*, *Livistona chinensis*, *Sabal adansoni* and *S. blackburniana* was obtained from the Indian Botanic Gardens, Calcutta. In all cases formalin-acetic-alcohol was used as the fixative. Transverse and longitudinal sections were cut from 4-12μ in thickness and stained with Delafield's haematoxylin or a combination of safranin and fast green. Due to the presence of a thick cuticle, abundance of sclerenchyma and raphides, some difficulty was experienced in microtoming.

INFLORESCENCE AND FLOWER

The spadix in *Livistona* and *Sabal* is lax and the branches are subtended individually by spathes. In *Pritchardia* it is once pinnate and the spikes are closely clustered and protected by a few basal spathes. In all species, the flowers

are bracteate, trimerous, regular and hermaphrodite. They are long pedicelled in *L. rotundifolia* (Fig. 1), shortly stalked in *Licuala grandis* (Figs. 17 and 18) seated on a pulvinate or tuberculate base in *Pritchardia* (Fig. 6) and *Trachycarpus*, (Figs. 43 and 44) and sessile in *Sabal* (Fig. 12). In *Livistona rotundifolia* the perianth is in the shape of a small 6-toothed cup out of which the essential organs emerge early and are protected by the smooth inner surface of the spathe. In other genera, the sepals are either free and imbricate as in *Trachycarpus* (Fig. 48), or united into a massive 3-toothed cup as in *Pritchardia* and *Licuala* (Figs. 9 and 17). The three petals are larger and valvate. In *Livistona rotundifolia* the stamens are free from the perianth and the anthers are basifixed (Fig. 1). In *L. chinensis*, *Pritchardia* and *Licuala* they are united into an epipetalous tube which in *Licuala*, splits into 6 teeth at the top (Figs. 5, 9 and 19); the anthers in these species are dorsifixed (Fig. 18). In *Livistona rotundifolia*, the gynoecium consists of a single uniovulate carpel; the margins of the carpel as well as the style are incompletely fused in the young condition (Figs. 2–4). In other genera including *L. chinensis*, there are 3 carpels which are free at the base (Figs. 5, 9 and 13); occasionally one carpel is suppressed (Fig. 14). The style of the young pistil is as thick as the ovary (Figs. 6 and 7); later due to greater growth in the ovary part it remains relatively slender in the mature pistil (Fig. 20). The three separate stylar arms fuse together in the upper part. The loculi extend as narrow canals into the stylar arms either from the top of the loculus as in *Pritchardia* (Fig. 8), *Trachycapus* (Fig. 16) and *Licuala grandis* (Fig. 21), or from the base as in *Sabal* (Fig. 12) and *Licuala chinensis* (Fig. 15). These locular canals may fuse with the common stylar canal (Figs. 10, 11 and 30), which is lined by either glandular cells (Fig. 29) or finger shaped hairs (Figs. 42 and 108). The stigma bears glandular hairs (Fig. 109).

For a thorough understanding of the floral structure and proper appreciation of the modifications undergone by the gynoecium in other tribes, a knowledge of the vascular anatomy of the flower is essential. That of *Licuala grandis* is described here as typical for the Sabaleae. The pedicel shows a number of 'fibro-vascular' bundles distributed irregularly in the central part (Fig. 22). At the base of the thalamus these bundles fuse together and form a plexus from the periphery of which a number of traces are given off for the calyx (Fig. 23). The central plexus now loses its sclerenchymatous mantle and gives off 3 arcs of traces for the petals. It then becomes triangular and gives off 6 staminal traces, the three antisepalous ones from the angles and the 3 antipetalous ones from the middle of the flat sides (Fig. 24). Thus the corolla and stamens show only congenital concrescence but not the adnation of their traces. The staminal and petal traces pass together into the androecium-corolla tube (Figs. 25 and 28). After the emergence of the staminal traces, the central stele breaks up into 3 rings of bundles, one for each carpel (Fig. 25). Three of the bundles at the base of the carpel bend inwards and enter the funicle (Fig. 26); these diverge in the raphe and branch slightly (Fig. 27). Though several carpellary bundles enter the base of the stylar arm, only three extend towards the top (Fig. 29 and 31).

ORGANOGENY

Floral organogeny has been followed in *Pritchardia pacifica*, *Sabal palmetto* and *Trachycarpus* sp. The floral organs arise in acropetal sequence, viz., bract., calyx, corolla, androecium and gynoecium. The bract and calyx become large before the other floral organs develop (Figs. 32–36; 43 and 44). The walls of the carpels arise as 3 bracket-like outgrowths, the margins of which remain separate for some time (Figs. 47). The floral axis enclosed by them is at first conical but soon becomes 3-lobed, each lobe being an ovule primordium (Figs. 37, 38, 45 and 46). After growing upwards for some time the tip of the carpellary

wall curves inwards and downwards, surrounds the ovule primordium and forms the loculus (Figs. 38 and 46). The upper parts of the carpels coalesce and grow together into the common style. In *Sabal palmetto* the loculus does not extend into the stylar arm; the incurved part of the carpellary wall touches the funicle which is transversely elongated, leaving only a small lateral opening through which the loculus communicates with the stylar canal (Figs. 39–41). In *Trachycarpus* and *Pritchardia* the descending portion of the carpellary wall grows to the base of the ovary and the loculus extends into the stylar arm as a narrow terminal canal (Figs. 45 and 46). By the time the archesporium is differentiated in the ovule primordium, the cells at the tip of the style become papillate; these develop into conspicuous hairs by the time the megaspore mother cell is full grown (Figs. 38 and 39). A number of cells of the ovary wall and style enlarge, lose their protoplasmic contents and become filled with bundles of acicular raphides (Figs. 39 and 41). In *Sabal palmetto* the wall of the mature ovary is uniformly parenchymatous; in *Licuala* sp. the cells of the outer half of the ovary wall accumulate tannin and those of the inner half become sclerified (Fig. 49).

MICROSPOROGENESIS AND MALE GAMETOPHYTE

The archesporium of the anther consists of one or two rows of hypodermal cells in each of the 4 anther lobes (Fig. 50). By a periclinal division they form the primary pareital cells to the outside and the primary sporogenous cells to the inside (Fig. 51). By further divisions in the parietal cells, the anther wall becomes 4–5 layered (Figs. 52–54 and 64). The epidermal cells in the mature anther become tangentially flattened and develop a thick cuticle which is ridged in *Sabal* sp. and *Pritchardia* (Fig. 67). The cells of the hypodermal layer enlarge considerably and develop fibrous thickenings. Sometimes the thickenings extend to two layers of cells (Fig. 77). The cells of the septum between the two loculi of an anther lobe are tangentially flattened and thin walled; the cells to the outside of the septum are small and thick-walled and constitute the stomium (Fig. 60). In the mature anther the septum shrinks and the loculi coalesce; the shrunken septum persists for some time after the dehiscence of the anther (Fig. 77). Cells of the connective contain tannin and raphides (Fig. 246).

The innermost one or two layers of wall cells function as the tapetum and 2–3 median wall layers become crushed. The tapetum is of the secretory type and shows 2-nucleate cells; in this respect it differs from the tapetum of Lemnaceae (Maheshwari, 1954) and Araceae (Jussen, 1928) in which it is plasmodial. The tapetal cells may be tangentially flattened as in *Sabal adansoni* (Fig. 61) or radially elongated as in *S. blackburniana* (Fig. 59), *Pritchardia* (Fig. 65) and *Washingtonia* (Fig. 70). In *Pritchardia* they penetrate into the mass of sporogenous cells and give the latter an irregular outline (Figs. 66 and 247). In *Trachycarpus* the tapetal cells store starch as reserve food (Fig. 76). In *Sabal palmetto* the inner walls of tapetal cells become cutinised (Fig. 58).

In all species, the sporogenous cells show a secondary increase (Figs. 53 and 64). In *Livistona rotundifolia*, some of the sporogenous cells degenerate before the meiotic divisions (Fig. 78). Microspore tetrads may be tetrahedral (Figs. 56 and 72) or bilateral (Figs. 55 and 71). The microspores become ellipsoidal by the time they separate out from the tetrads and show a distinct furrow (Fig. 73). In *Washingtonia* sometimes all the pollen tetrads of an anther loculus become degenerate and the loculus becomes occluded by the intruding tapetal cells (Fig. 70).

Cytokinesis of the sporocyte is of the simultaneous type and is brought about by cell plate formation at the end of meiosis II (Fig. 62). The small lenticular generative cell is usually formed against the wall opposite to the furrow (Figs. 57 and 74), and later it migrates into the vegetative cytoplasm. The generative

cytoplasm is hyaline and devoid of starch while the vegetative cytoplasm is packed with it.   In the full grown pollen grain the generative cell appears crescent shaped with tapering ends; it is circular in transverse section (Figs. 63, 68 and 75).   A similar shape is reported in several Asclepiadaceae (Finn, 1925; C. V. Rao and S. R. Rao, 1954).   The pollen grains are shed in the 2-celled condition in which respect they resemble the Araceae (Banerji, 1947).   In all species they are monocolpate and aporate, and show a smooth exine (Fig. 79).   The exine does not stain with Delafield's haematoxylin except in the region of the furrow; this shows that the region of furrow is chemically different from the rest of the exine which fact may be associated with its slight extensibility.   Sometimes in mature pollen grains the intine bursts through the furrow and forms a short blunt pollen tube *in situ* (Fig. 69).   Sterile pollen grains are commonly noticed in all species.

OVULE

In all species studied, the ovules are erect, bitegmic and crassinucellate.   In *Livistona rotundifolia* (Figs. 87, 88 and 254) and *Sabal* sp. (Figs. 99, 102, 116, 121, 123 and 252), they are hemianatropous.   In *L. chinensis* (Figs, 94 and 253), *Pritchardia* (Figs. 136 and 248), *Washingtonia* (Figs. 147 and 152), *Licuala* sp. (Figs. 162, 167, 173, 174, 178 and 179) and *Trachycarpus* (Fig. 185) they are anatropous. Two or three vascular bundles enter the funicle of the ovule (Figs. 26, 117 and 176) and branch slightly in the raphe and chalaza (Figs. 139 and 167).   In *Livistona rotundifolia*, however, a transverse section of the funicle shows a ring of 6-7 vascular bundles (Fig.89) which show further branching in the body of the ovule (Figs. 90-93). Eames and Macdaniels (1947) remark, "when an ovule represents the surviving member of a group in which reduction has occurred, it may have captured the trace supply of two or more ovules".   It may be recalled here that in *Livistona chinensis* the pistil consists of three uniovulate carpels while in *L. rotundifolia* it shows a single uniovulate carpel.

The different species studied show interesting stages in the evolution of a funicular obturator.   In *Pritchardia*, and *Washingtonia*, the funicle is unspecialised. In *Licuala peltata* (Fig. 174) *L. spinosa* (Fig. 176) and *Trachycarpus* (Fig. 185), the epidermal cells of the funicle are somewhat radially elongated and glandular.   In *Livistona chinensis* and *L. rotundifolia* the funicle develops a basal swelling, the epidermal cells of which are markedly elongated and glandular (Figs. 87, 89–92, 94). In *L. chinensis* the locular canal and micropyle adjoin the obturator (Fig. 15). The obturator in *Sabal* sp. is very conspicuous.   In *S. palmetto* the epidermal cells of the funicle form finger-shaped, 1-celled glandular hairs (Figs. 115–117 and 249).   In *S. adansoni* and *S. blackburniana*, due to the divisions in the epidermal cells, a considerable amount of glandular tissue is formed (Figs. 100, 103–107, 123, 250–252).   The superficial cells of this tissue form elongated hairs.   Similar hairs are developed from the locular epidermis at the back of the ovule and these come into intimate contact with the cells of the obturator (Fig. 105).   The stigmatic hairs, glandular cells of the transmitting tissue and the obturator form a continuous path of richly protoplasmic cells for the pollen tubes.   The obturator in *Sabal* sp. and *L. rotundifolia* (Fig. 225) persists till the early stages of seed development.

A prominent growth of the chalazal region is the characteristic feature of all genera.   A comparison of the figures of the ovules with megaspore tetrads and full grown embryo sacs will bring out this point (cf. 132 and 136).   The integuments are free from each other only for a small distance around the micropyle of the ovule.   In *L. rotundifolia* part of the growth in the chalazal region seems to be brought about by the periclinal divisions in the epidermal cells, which is evident from the regular arrangement of the derivatives (Figs. 91–93).   Accumulation of tannin in cells of the chalaza and outer integument, which commences even

from the tetrad stage of the ovule, is another feature common to all species (Figs. 132 and 136).

The initials of both the integuments become demarcated simultaneously with the differentiation of the archesporium in the ovule (Figs. 80, 81, 96, 110, 126, 127, 181 and 182). The rates of growth of the two integuments and the method of formation of the micropyle vary in the different species. In *L. rotundifolia,* *Sabal* sp. (Fig. 122) and *Pritchardia* (Fig. 134), the micropyle is formed by the 2- or 4-nucleate stage of the embryo sac. In *Washingtonia* (Figs. 141 and 143) and *Licuala* sp. (Figs. 158, 159), the micropyle is formed by the time the megaspore mother cell in the ovule is full grown. In *Sabal* (Figs. 101 and 123) and *Washingtonia* (Figs. 151 and 152), the micropyle of the mature ovule is formed only by the inner integument and is straight. However, after fertilization, in *Sabal* sp. also the outer integument grows over the inner and forms the micropyle (Figs. 215–217). In species in which the micropyle is formed by both the integuments, it may be short and straight as in *L. rotundifolia* (Figs. 88 and 93) or elongated and zigzag as in *Livistona chinensis* (Fig. 94), *Pritchardia* (Fig. 136), *Licuala* sp. (Figs. 162, 167, 168, 173, 174, and 179) and *Trachycarpus* (Fig. 185).

In general, the outer integument is 4–5 layered and the inner 2–3 cells thick. In nearly all genera studied, an integumentary tapetum is organised. In *L. rotundifolia* a glandular endothelium is not found in the mature ovules. The cells of the inner integument, however, become radially elongated after fertilization (Fig. 224). In *Sabal adansoni* the cells of the inner epidermis of the inner integument in the mature ovule are radially elongated though they are not glandular (Fig. 123). In genera which show endothelium, the concerned cells become markedly elongated and glandular even by the dyad stage of the ovule (Figs. 130, 143, 158). Due to the prominent endothelium the inner integument equals or even excels the outer in thickness. Though usually only the inner layer of the integument is glandular, in *Licuala* sp. both layers consist of such cells (Figs. 170, 177). In *L. grandis* the endothelial cells accumulate starch from an early stage of the ovule (Figs. 162 and 166); in *Pritchardia,* starch appears in the endothelial cells after fertilization (Fig. 190). Even in sterile ovules, the integuments, micropyle and endothelium are formed normally (Figs. 95, 140, 154, 155, 171 and 175).

Though the ovules are crassinucellate, the size of the nucellus is small relative to that of the ovule (cf. figs. 144, 163 and 172). In *L. rotundifolia* the mature embryo sac is surrounded by 3–4 layers of nucellar cells some of which persist till the early stages of seed development (Figs. 88 and 224). In *Sabal* sp. 1–2 layers of nucellus surround the mature embryo sac (Figs. 99, 116, 123 and 124). In species in which an endothelium is organised, the whole of the nucellus is crushed out in the mature ovule and the embryo sac borders on the inner integument. In *Sabal* sp. the nucellar cells around the antipodal end of the sac are thin walled and show vacuolated cytoplasm and seem to be tapetal in function (Figs. 123 and 124).

In *L. rotundifolia* (Fig. 88), *L. chinensis* (Fig. 94) and *Sabal* sp. (Figs. 99 and 123) no thick walled nucellar cells are found around the embryo sac; the sac is therefore even in outline. In *Pritchardia* (Fig. 136) and *Trachycarpus* (Fig. 185) the nucellar cells around the antipodal end of the sac are thick walled though they do not form a postament. In *Washingtonia* and *Licuala* sp. the nucellar cells around the lower half of the developing embryo sac become thick walled. As the sac enlarges, the thin walled nucellar cells around the micropylar part of the sac become gradually pulled apart from the thick walled cells and are absorbed in due course (Figs. 153, 165 and 166). The socket of thick walled cells persists and forms the postament (Figs. 152, 167, 168, 174, 178 and 179). Since the embryo sac expands at first on the sides of the postament, it has a sagittate appearance (in l.s.).

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

In all species, the embryo sac develops according to the *Normal*-type. The archesporium of the ovule consists usually of a single hypodermal cell (Fig. 110), two cells being noticed rarely (Fig. 181). It divides periclinally and forms the primary parietal cell to the outside and the megaspore mother cell to the inside (Figs. 81, 111, 127 and 182). This division occurs very early and the ovule primordium with megaspore mother cell grows for a considerable time (compare figs. 126 and 128). To get at the stage shown in fig. 126 of *Pritchardia*, a very small inflorescence still concealed among the leaf bases had to be obtained. It is probably due to the difficulty of getting such material that some investigators like Quisumbing and Juliano (1927) thought that the archesporium is subhypo-dermal and that it functions directly as the megaspore mother cell without cut-ting off the primary parietal cell. By the division of the primary parietal cell and its derivatives, 1-3 layers of parietal tissue are formed (Figs. 96, 114 and 132). In *Livistona rotundifolia*, however, the parietal tissue remains uniseriate and the megaspore mother cell becomes deep seated due to the development of a nucellar cap (Figs. 82–86).

The full grown megaspore mother cell is elongated and tapering (Figs. 97, 112, 118, 119, 131, 142, 157 and 183). Both dyads derived from it may divide simultaneously or one may precede the other (Figs. 86, 113, and 145). Megaspore tetrads are usually linear (Figs. 85, 114, 120, 122, 146, 147–149, 160 and 161), though T-shaped and ⌐-shaped tetrads are occasionally met with (Figs. 98, 132, 133 and 150). Kajale (1952) also reported different types of tetrads in *Elaeis guinensis*.

The lowest megaspore always functions and gives rise to the 8-nucleate embryo sac. The synergids show hook-like or rounded protuberances on their free sides (Figs. 180 and 187). The polar nuclei fuse before fertilization; the secondary endosperm nucleus stands close to the egg apparatus (Fig. 101) or at the middle of the sac (Fig. 88) or nearer to the antipodals (Fig. 94). The antipodals are 3 in number and uninucleate (Figs. 125 and 169). In *Pritchardia* they are small when formed but enlarge considerably and show starch at maturity (Figs. 136, 138). Mature embryo sac may be ellipsoidal in shape as in *L. rotundifolia* and *Sabal* sp. or may show a narrow antipodal end and broad micropylar part as in *Licuala* sp. or narrow micropylar and broad antipodal parts as in *Livistona chinensis* (Fig. 94).

ENDOSPERM, EMBRYO AND SEED DEVELOPMENT

Fertilization is porogamous and one or both the synergids become affected by the entry of the pollen tube into the embryo sac (Figs. 190 and 218). By the time syngamy occurs, a few endosperm nuclei are formed. The endosperm is of the nuclear type. After fertilization the protoplasm of the embryo sac and the starch in it increase in quantity (Fig. 191). The endosperm becomes cellular by simultaneous cell plate formation which commences at the periphery of the sac. Cell walls are incomplete towards the central cavity of the seed (Fig. 242). The endosperm grows centripetally by intercalary cell divisions which are accompanied by cell wall formation (Fig. 229). Since the cells are laid in regular radial rows (Figs. 197 and 228), the palm endosperm cleaves so readily and so perfectly. In *Pritchardia* and *Sabal* the endosperm is entire but in *Licuala* sp. it becomes divided into islands or pockets by chalazal ruminations. In each such zone, the regular arrangement of the endosperm cells can still be seen (Fig. 241). The endosperm cells at the periphery of the seed are smaller and more richly protoplasmic than those of the inside (Figs. 228 and 229). Reserve food materials are accumulated in the lumens of endosperm cells. Gradually the walls of the cells become massive and pitted due to the deposition of hemicellulose (Figs. 198 and 221). In *Sabal*

palmetto the developing seed does not grow symmetrically all over; the micropyle therefore becomes shifted in position away from the funicle (Figs. 215 and 216).

Pritchardia, Sabal, Livistona and *Licuala* in which seed development has been studied, show increasing elaboration of the chalazal region. In *Pritchardia* the fertilised ovule grows rapidly first in length and then in diameter till it becomes round (Figs. 188 and 189). The sac, including the antipodal end, widens out. During the early stages of seed development there is considerable proliferation of the cells surrounding the lower part of the sac. The derivatives which stand in regular series, sometimes form wavy protruberances into the embryo sac and appear as incipient ruminations (Fig. 196). The cells of the chalaza and integuments accumulate tannin; the former persists as a ridge in the seed opposite to the embryo (Fig. 197). The coat of the mature seed becomes even in outline; it consists of 10–12 layers of cells most of which are filled with tannin (Fig. 198).

In *Sabal palmetto* the chalazal cells around the lower part of the sac accumulate tannin and become thick walled; this part persists as a pit like depression even in the mature seed (Figs. 216, 219 and 220). The endosperm in this species is not pierced by any ruminations.

During seed development in *L. rotundifolia* the cells of the chalaza become thick walled and filled with tannin. The seed grows mostly in the chalazal region; the embryo sac expands to the sides of the chalazal protuberance (Fig. 225) which first appears as a massive cup and then grows through the seed cavity as a thick tube. Vascular bundles extend into it and branch slightly (Fig. 226). In the mature seed it appears as a dark coloured columnar structure bridging the whole diameter of the seed (Fig. 227). No ruminations develop from it or the seed coat so that the endosperm is even in outline (Fig. 228). The seed coat in this species consists of 5–7 layers of cells of which the two outermost are free from tannin (Fig. 229).

As in *Pritchardia*, in *Licuala* also, the fertilised ovule grows first in length and then in diameter till it becomes round (Figs. 237–240). The postament breaks down and the embryo sac widens out. The tannin filled cells of the chalaza form first a hump-like protuberance into the sac (Fig. 237). This does not show any pit like depression as in *Sabal* and *Livistona*. Proliferations develop from its surface and branch and grow till some of them touch the wall of the seed cavity (Figs. 238–240). Vascular bundles of the funicle extend into it and branch within these proliferations. The endosperm in *Licuala* is therefore ruminate in the true sense of the word, though the ruminations develop from the chalaza and not from the seed coat as in *Areca catechu*. The seed coat in *Licuala* is even in outline and consists of 7–8 layers of tannin filled cells (Fig. 242).

Only a few stages of the embryo development could be obtained in *Pritchardia pacifica*. The fertilised egg divides transversely and gives rise to *ca* and *cb* (Figs. 192 and 193). The first division in *ca* is oblique (Fig. 194). The derivatives of *ca* give rise to embryonal mass while those of *cb* form the suspensor which is relatively massive (Fig. 195). Similar stages were also noticed in the embryo development of *Areca catechu* (C. V. Rao, 1955).

Mature embryo in *Pritchardia* measures about 5 mm long and is cylindrical in the lower part and conical in the upper. The primary axis which is confined to the lower part is relatively small. The massive lateral cotyledon envelops it completely leaving a pore for its emergence during germination (Fig. 199). The hypocotyl shows a ring of procambial strands from which branches are given off into the cotyledon. These traverse nearly to the tip of the cotyledon forming a ring near the periphery (Figs. 200 and 201). The cells of the cotyledon are packed with food materials (Fig. 202). The cells of the cotyledonary sheath immediately below the radicle are thin walled and devoid of starch; this region is pierced first by the elongating radicle during germination. The plumule shows number of a scale and leaf primordia (Fig. 208).

FRUIT AND GERMINATION OF SEED

Usually only one of the carpels forms the fruit in *Pritchardia* and *Sabal* but in *Licuala* sometimes all the three carpels may form fruitlets (Figs. 211, 215, 235 and 236). The calyx and style are persistent in *Pritchardia* and *Livistona* (Figs. 211, 226 and 227). Some of the cells of the style become sclerenchymatous.

The fruit wall shows some variation in structure in the different species. In *L. rotundifolia*, the ovary wall shows vascular bundles in the median region and a zone of 10–12 layers of parenchyma on either side. The cells to the outside are small while those to the inside are larger, scantily cytoplasmic and some bear raphides. In the fruit wall 2–3 layers of cells to the outside of the vascular bundles develop into sclereids (Fig. 234). The parenchymatous cells to the inside become shrunken and those at the periphery develop into a succulent pericarp. In *Pritchardia* the ovary wall does not show any sclerenchyma (Fig. 212). In the fruit wall two zones of sclereids develop, one on either side of the vascular bundles. Some more sclereids are found interspersed among the parenchyma and a sheath of sclerenchyma is found around the vascular bundles. As the thick walled cells are oriented in different directions, the stony pericarp in this species is well adapted to resist crushing strains (Figs. 213 and 214). In *Licuala grandis* the ovary wall shows a zone of sclerenchyma beneath the surface (Fig. 243); in the fruit wall another zone of sclerenchyma is added towards the inside (Fig. 244). The sclerenchyma as well as the parenchyma show sphaeraphides (Fig. 245).

In *Pritchardia* the position of the embryo in the seed is marked by a circular depression just by the side of the raphe (Figs. 203 and 204). During germination of the seed in the species studied, the cotyledon forms a long or short tube which bears at its tip the primary axis (Figs. 205–207, 222, and 230). The plumule emerges through the sheath; the radicle forms an elongated root which eventually becomes arrested and is replaced by adventitious roots (Figs. 209, 223, 231–233).

ACKNOWLEDGEMENTS

REFERENCES

Bailey, L. H. (1930–1949). Several papers on systematics of American and cultivated palms in *Gentes Herbarium*, Vols. 2, 3, 4, 6, 7 and 8.

Banerji, I. (1947). Life history of *Typhonium trilobatum* Schott. *Proc. Nat. Inst. Sci. Ind.* **13.**, 207–230.

Bentham, G. and Hooker, J. D. (1862–1883). *Genera Plantarum*, London.

Blatter, E. (1926). Palms of British India and Ceylon. Oxford Univ. Press.

Bosch, E. (1947). Blutenmorphologie und zytologische untersucheingen an Palmen. *Schw. Bot. Ges.* **57**, 37–100.

Dahlgren, K. V. O. (1936). Index of American palms. *Field Mus. Nat. Hist. Bot. Studies.* Vol. 14.

De Poerck, R. A. (1950). Contributions a l'etude du Palmier a huile African *Elaeis guinensis* Jacq. *Oleagineux*, **5**, 623–62.

Drude, O. (1895). Classification of Palmae in Engler's *Naturlichen Pflanzenfamilien*.

Eames, A. J. and MacDanials, L. H. (1947). *An Introduction to Plant Anatomy*. McGraw Hill Co. N.Y.

Engler, A. and Prantl, K. (1895). Naturlichen Pflanzenfamilian. Leipzig.

Finn, W. W. (1925). Male cells in Angiosperms. Spermatogenesis and fertilization in *Asclepias cornuti*. *Bot. Gaz.* **80**, 1–25.

Hutchinson, J. (1934). *Families of Flowering plants. II. Monocotyledons.* Macmillan.

Juliano, J. B. (1931). Morphology of the male flower of *Cocos nucifera* L. *Phillippine Jour. Sci.* **45** (3), 449–458.

Jussen F. J. (1928). Die Haploidgeneration der Araceen und ihre Verwertung fur das System. *Englers Jahrb* **62**, 155–283.

Kajale, L. B. (1952). Occurrence of four kinds of tetrads in *Elaeis guinensis* Jacq. *Curr. Sci.* **21**, 170.

Kajale, L. B. and Ranade, S. G. (1953). The embryo sac of *Elaeis guinensis* Jacq. A reinvestigation. *J. Ind. Bot. Soc.* **32**, 101–107.

Lawrence, G. H. M. (1955). Taxonomy of vascular plants. Macmillan.

Maheshwari, S. C. (1954). The embryology of *Wolffia. Phytomor.* **4**, 355–365.

Martius, C. F. P. (1923–1950). Von Historia Naturalis palmen 3 Vols. Leipzig.

Quisumbing, F. and Juliano, J. B. (1927). Development of the ovule and embryo sac in *Cocos nucifera* L. *Bot. Gaz.* **84**, 279–293.

Rao, C. V. (1955). Embryo development in arecanut. *Nature* **175**, 432.

Rao, C. V. and Rao, S. R. (1954). Embryology of *Cryptostegia grandiflora* R. Br., and *Caralluma attenuata* Wt. *J. Ind. Bot. Soc.*, **33**, 453–472.

Rendle, A. B. (1953). Classification of flowering plants. Vol. 1. Monocotyledons. Cambridge.

Schnarf, K. (1931). Vergleichende Embryologie der Angiospermen. Berlin.

Selvaratnam, E. M. (1952). Embryo of arecanut. *Nature.* **169**, 714.

Swamy, B. G. L. (1942). Embryological studies in Palmae. A preliminary note on the megasporogenesis in *Areca catechu* L. *Curr. Sci.* **11**, 109.

TEXT-FIG 1.

TXTE-FIG 2.
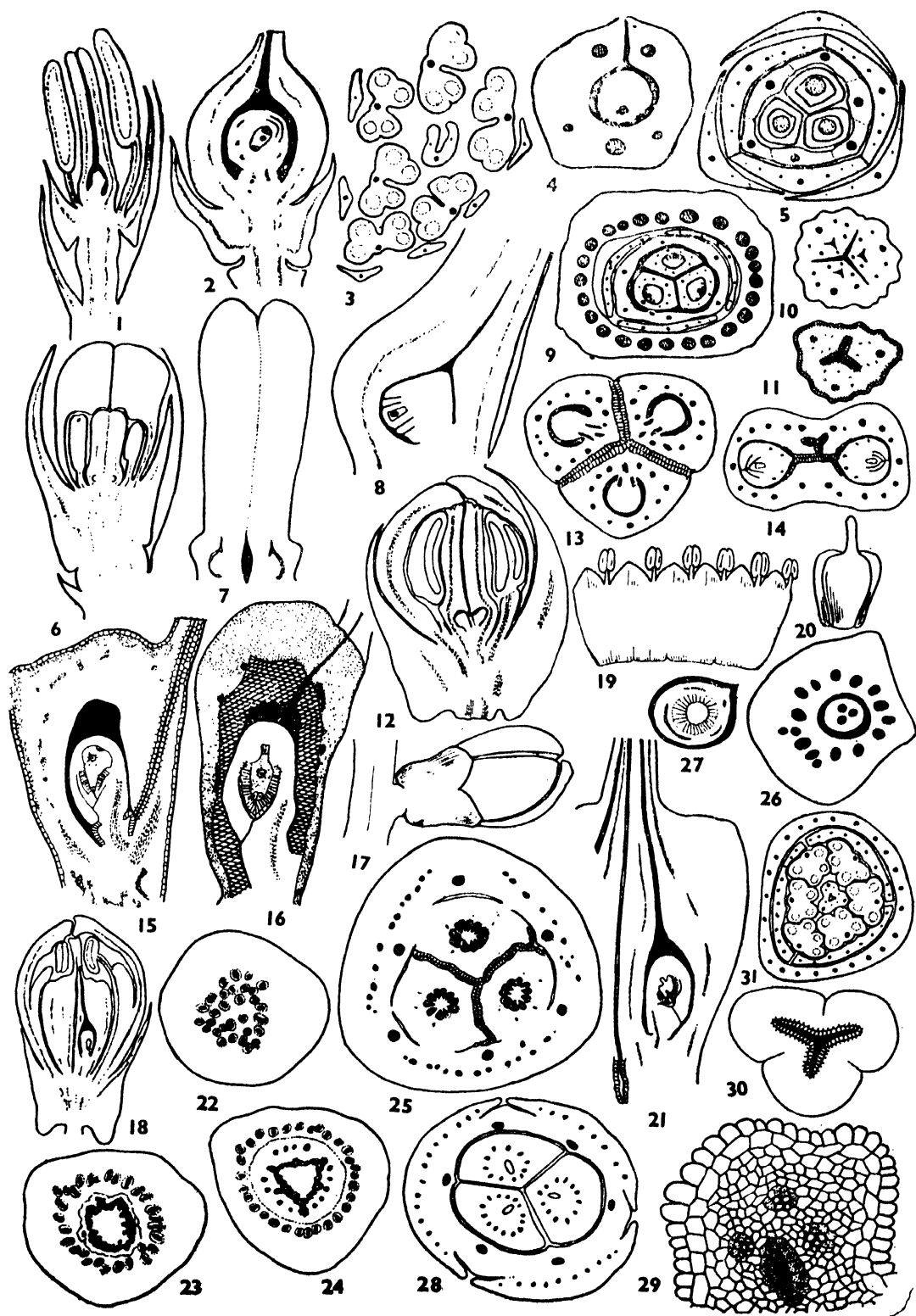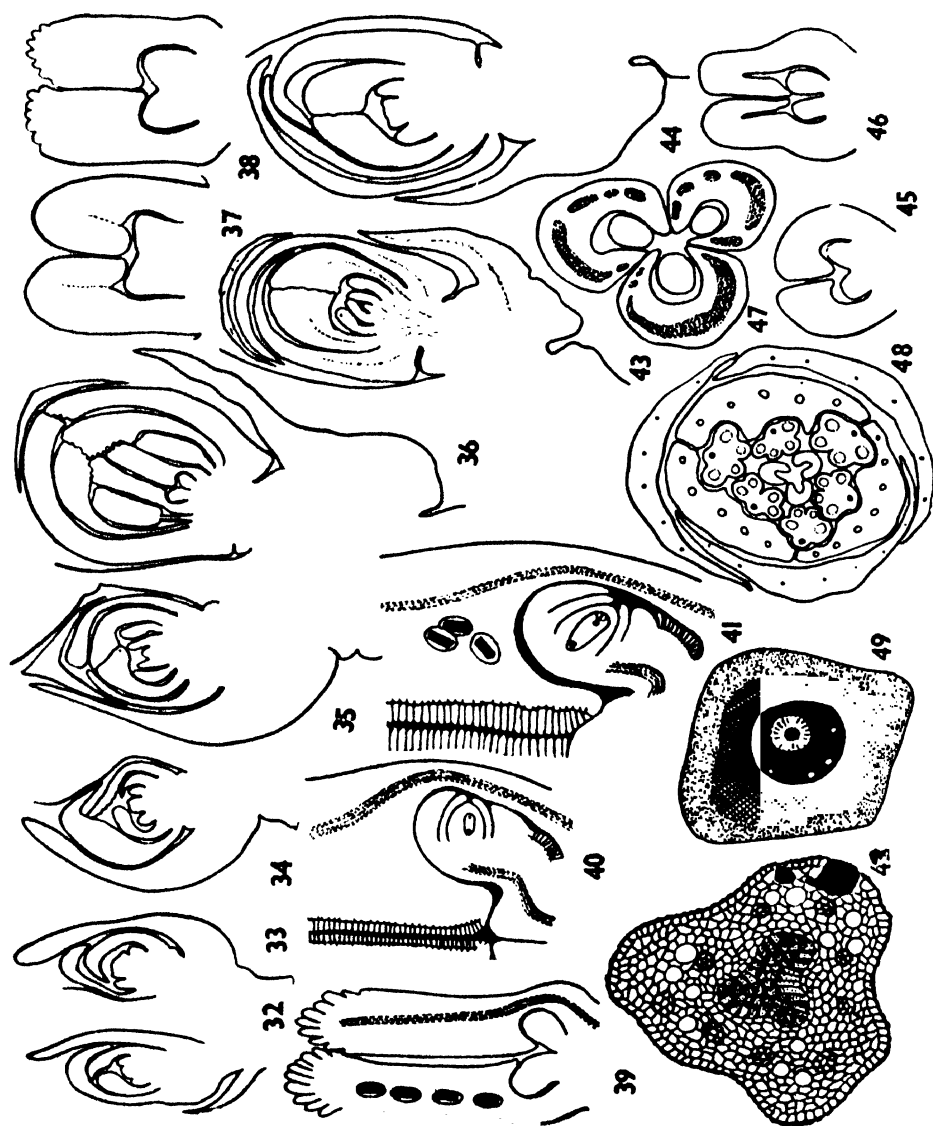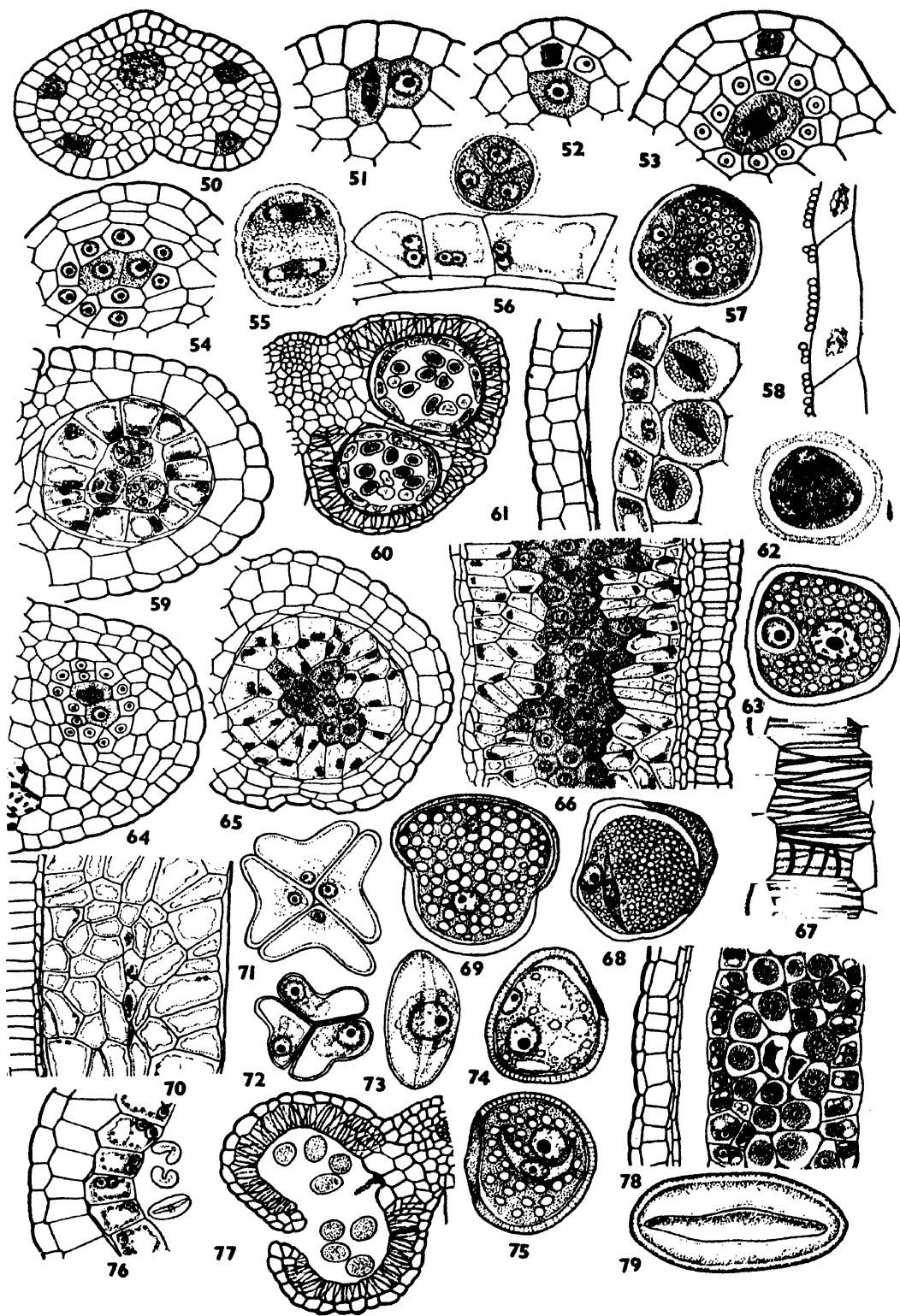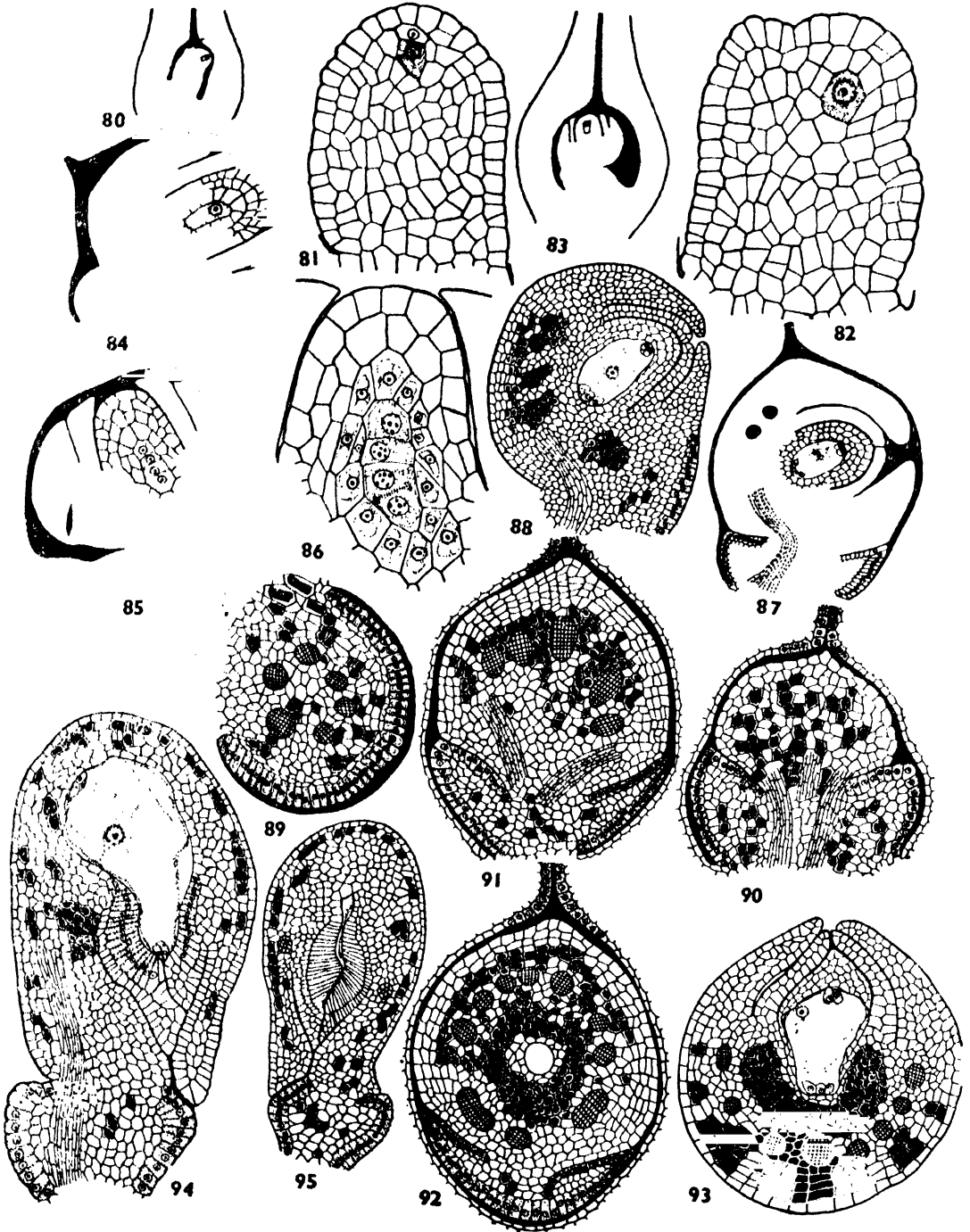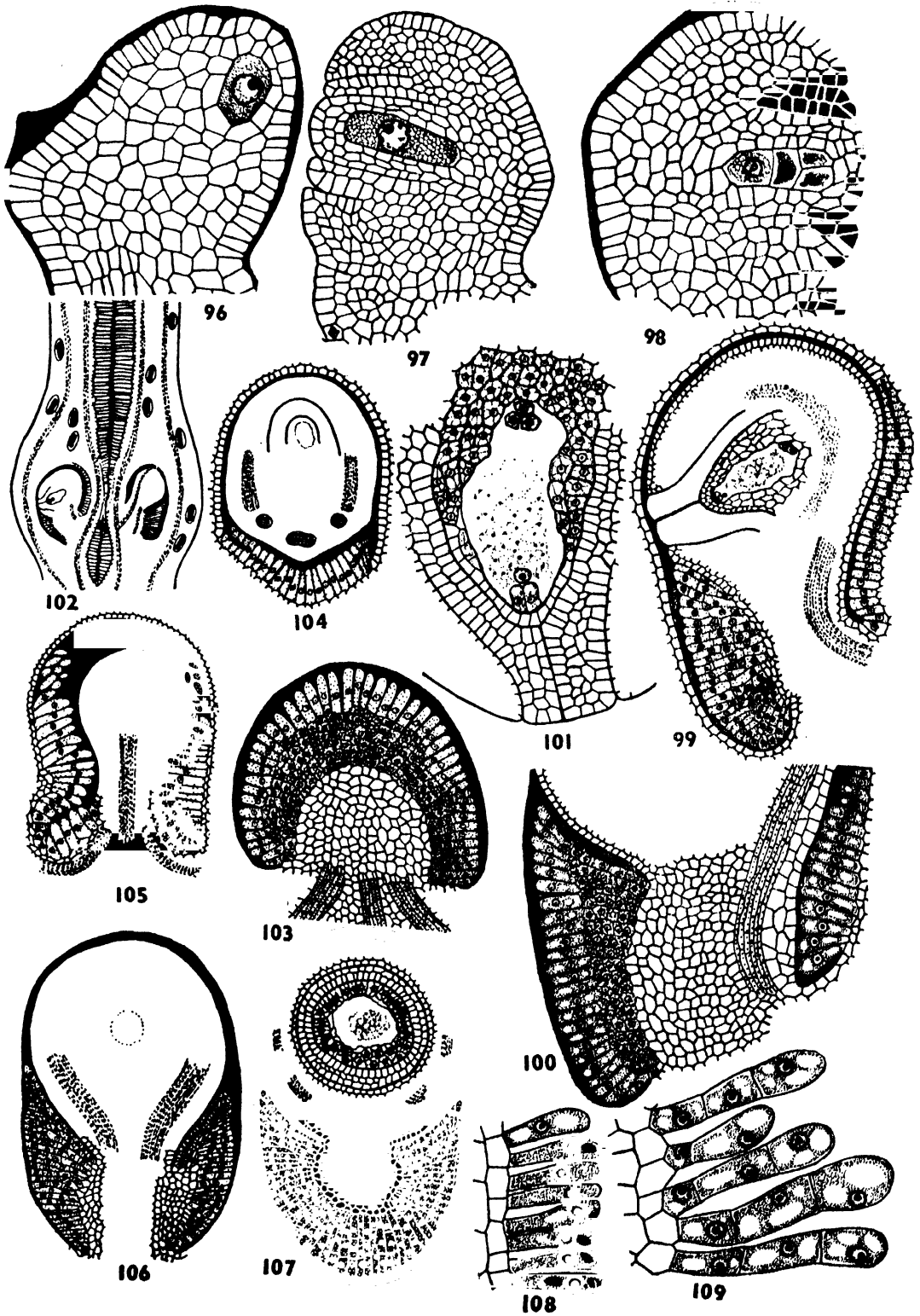
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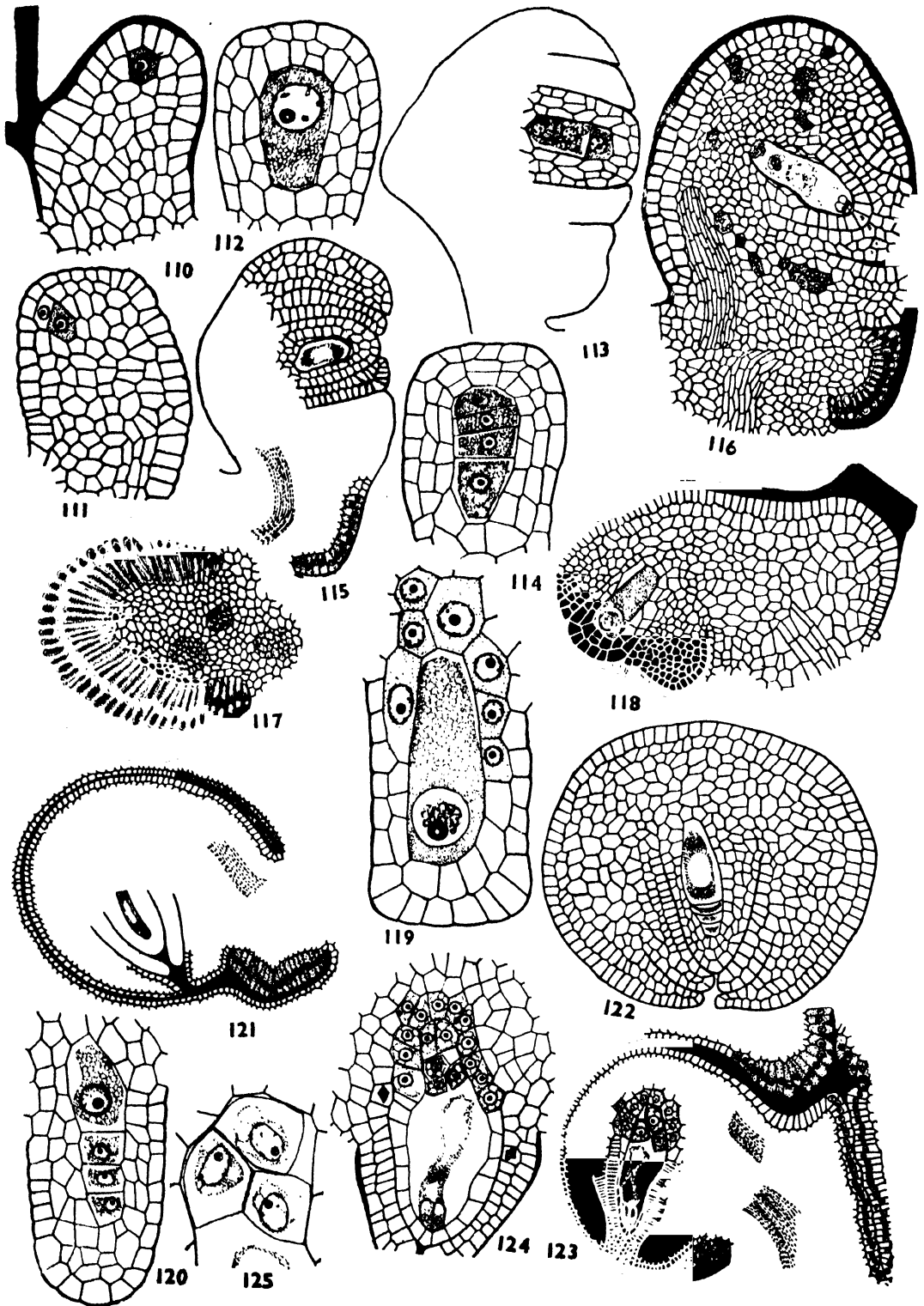
TEXT-FIG. 6

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TEXT-FIG 9

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TEXT-FIG. 10

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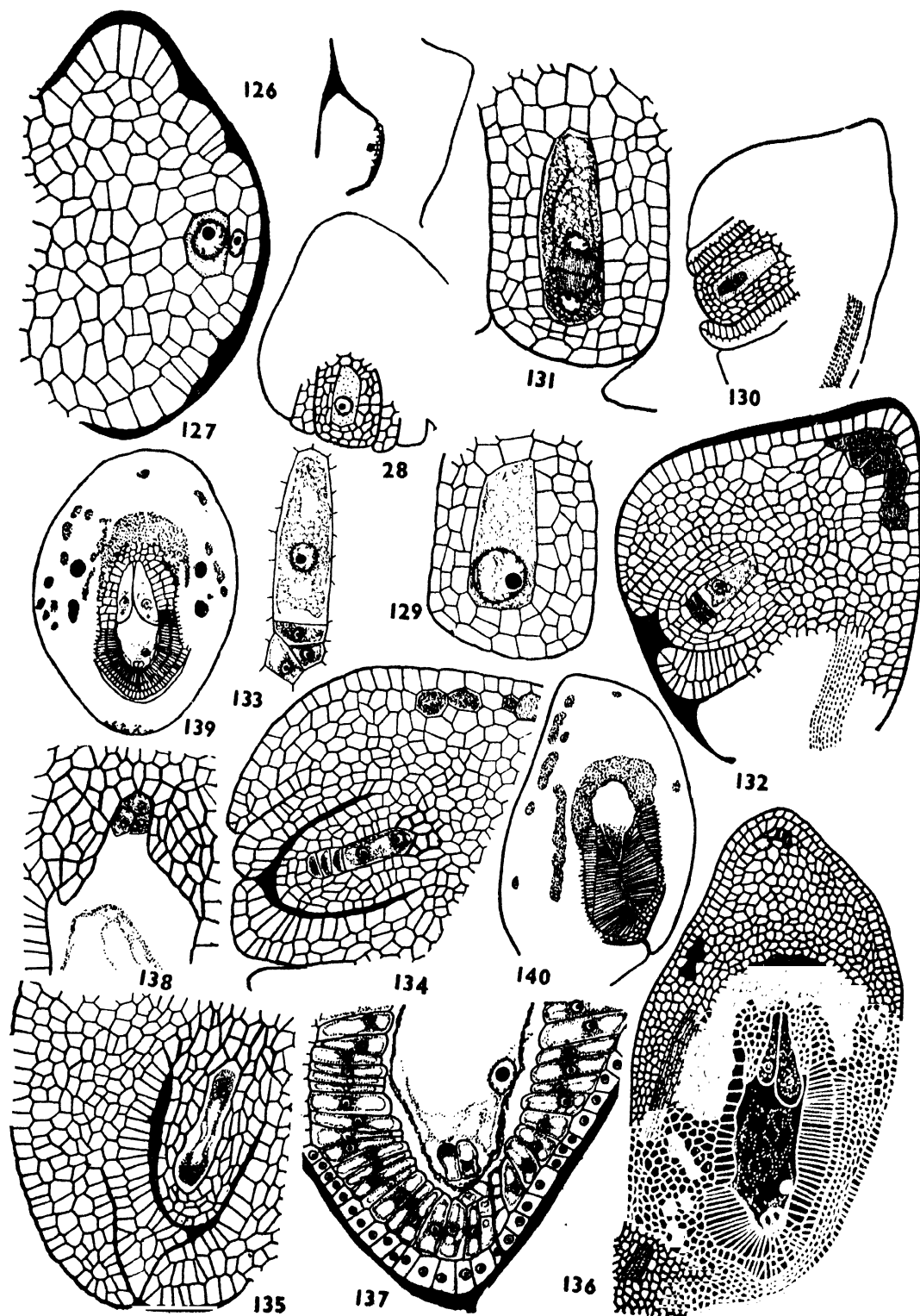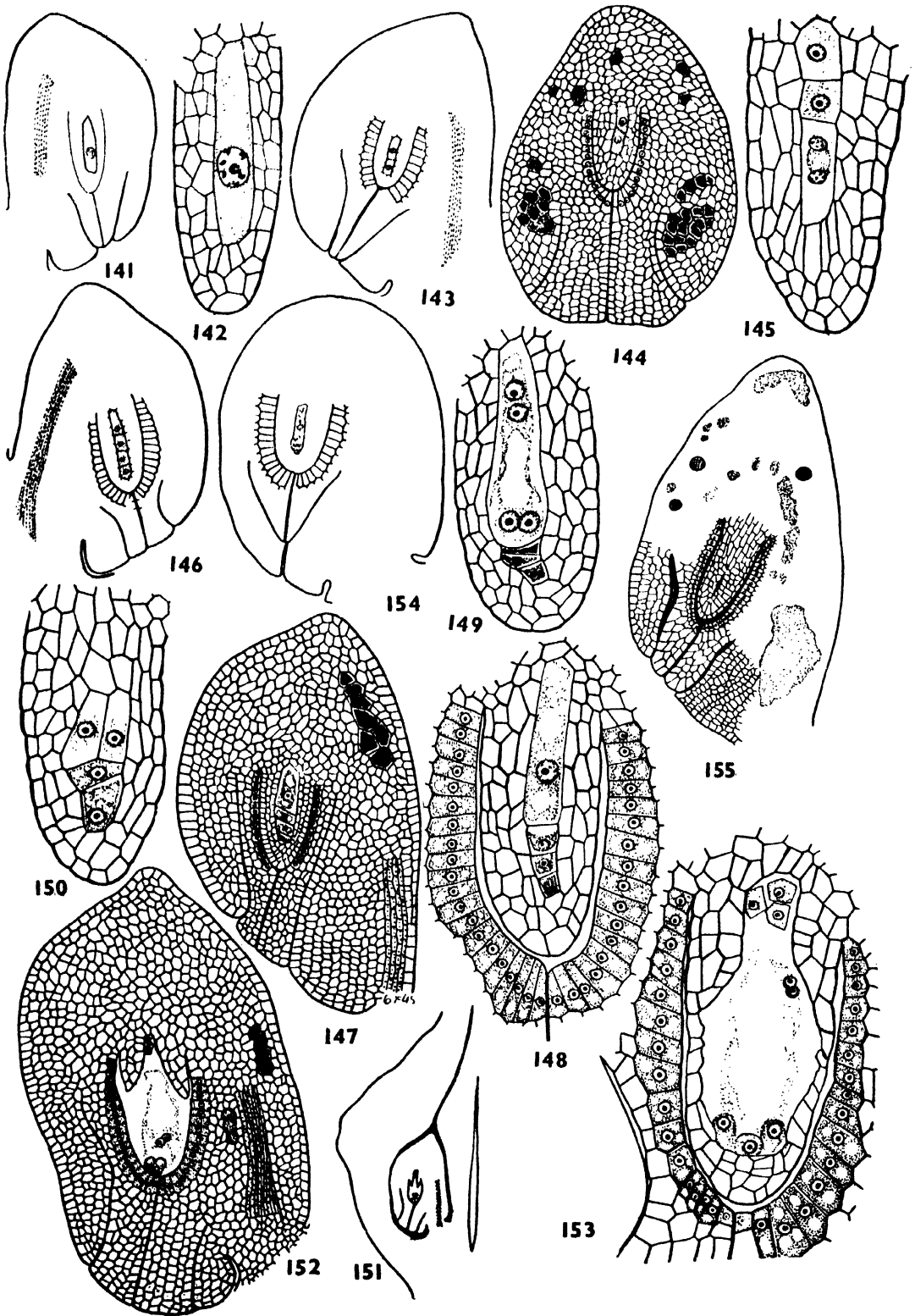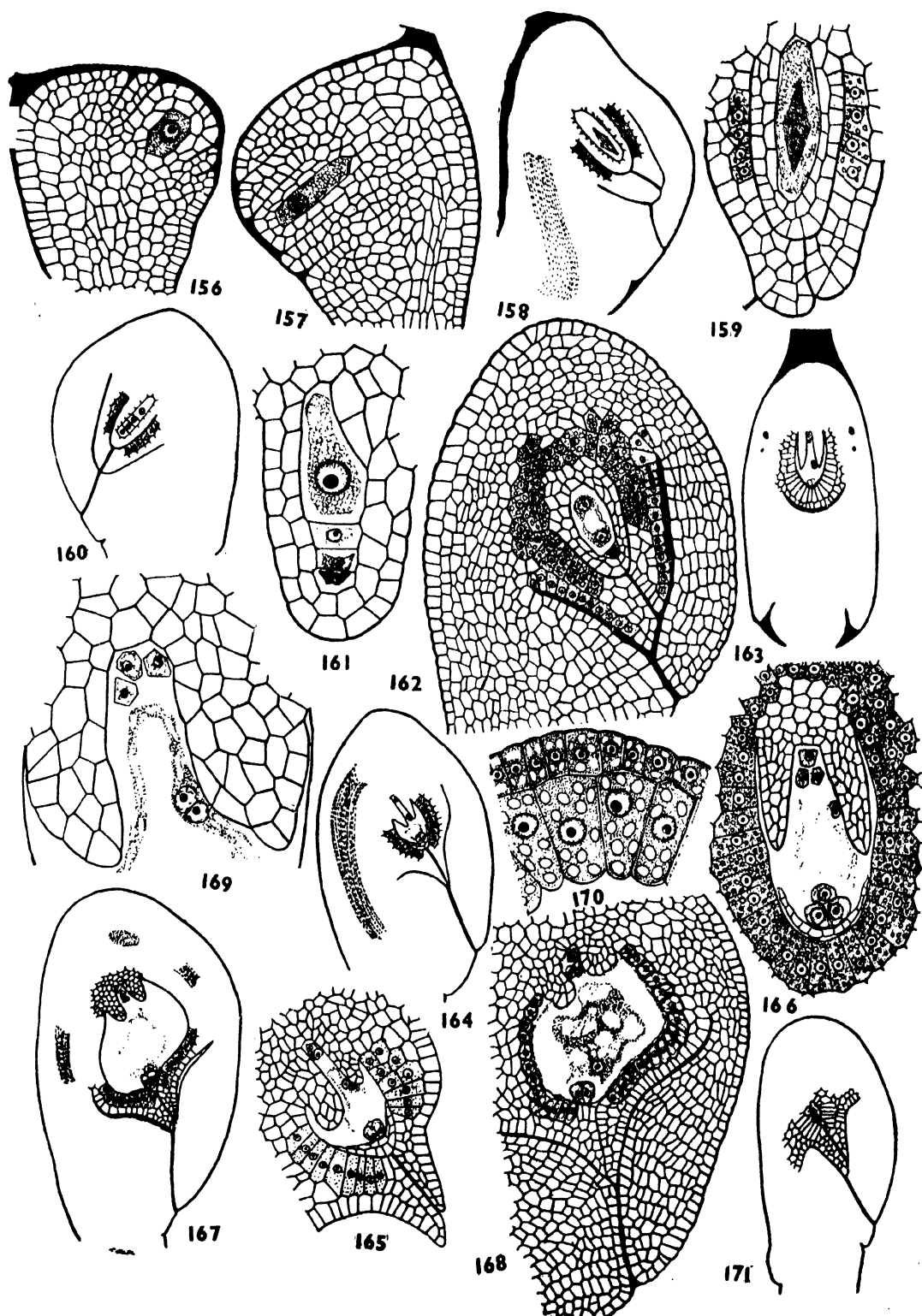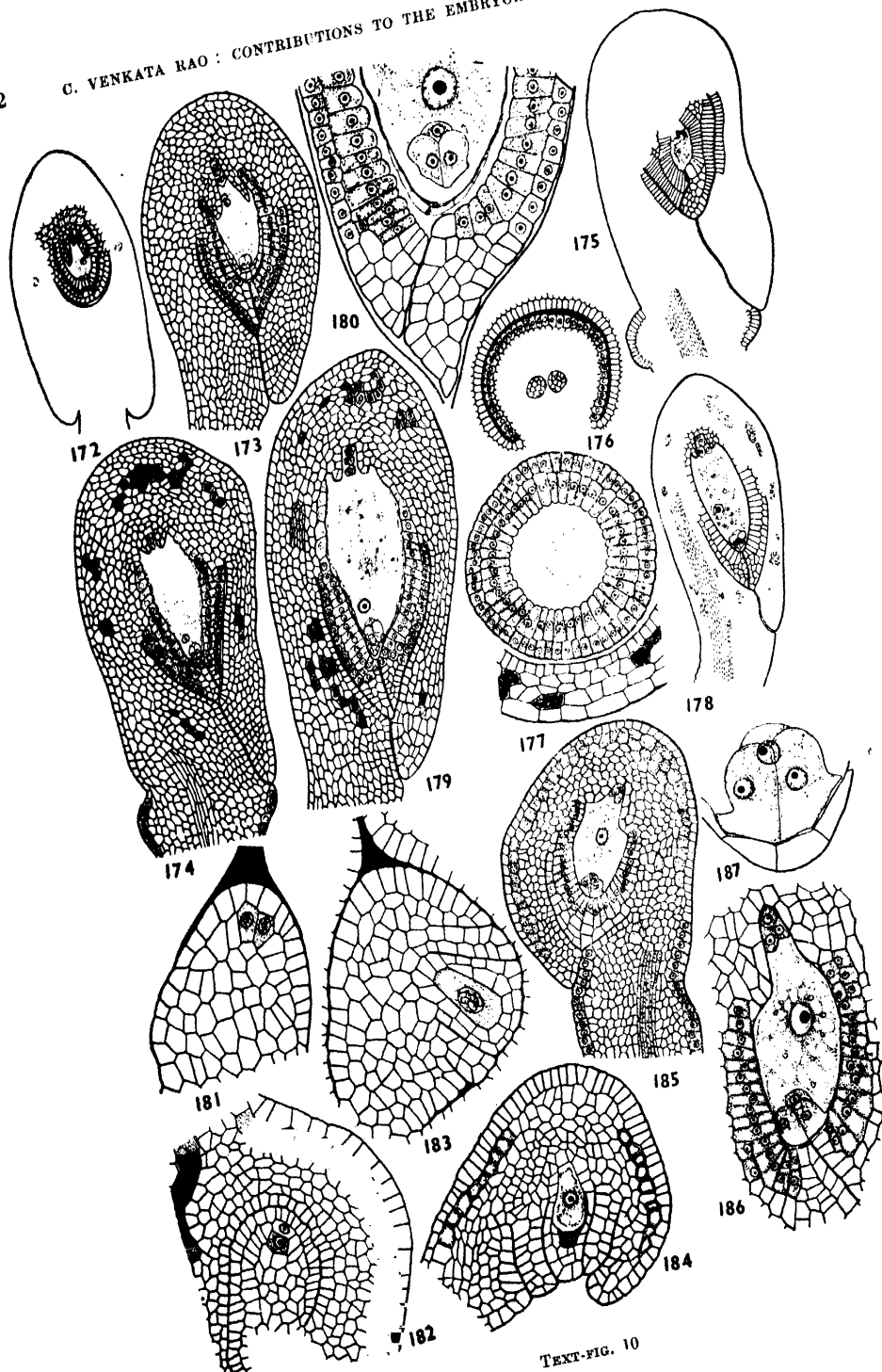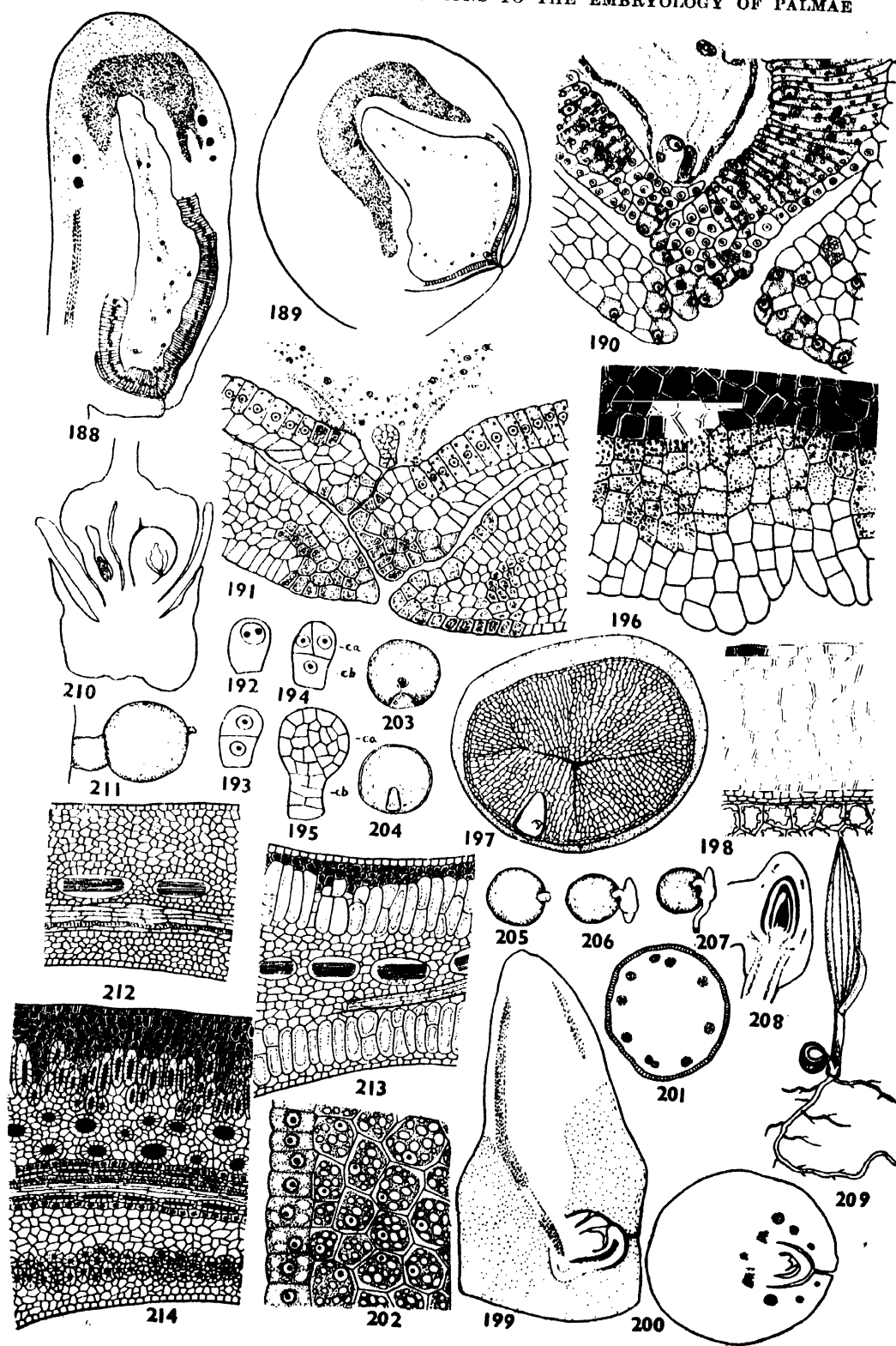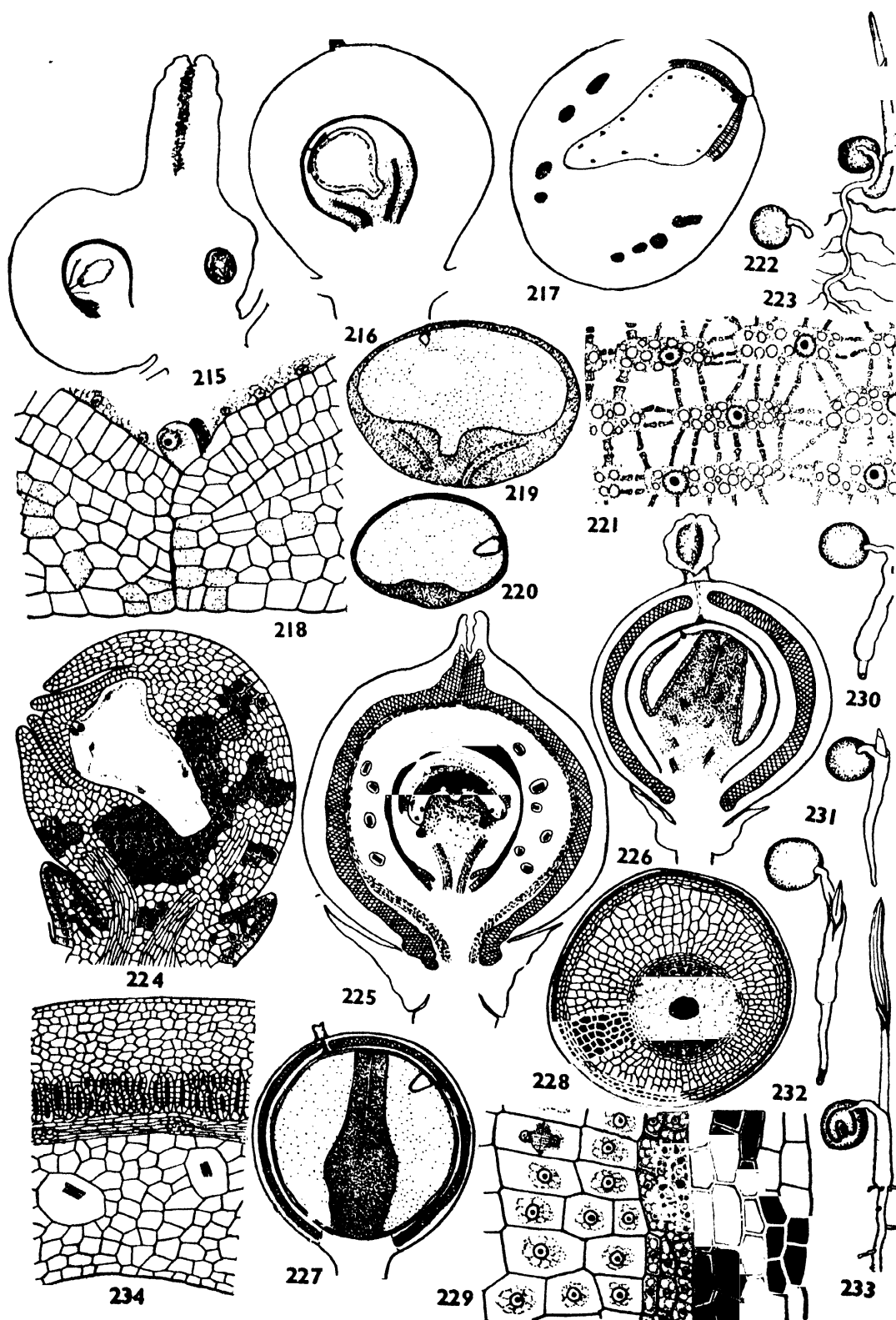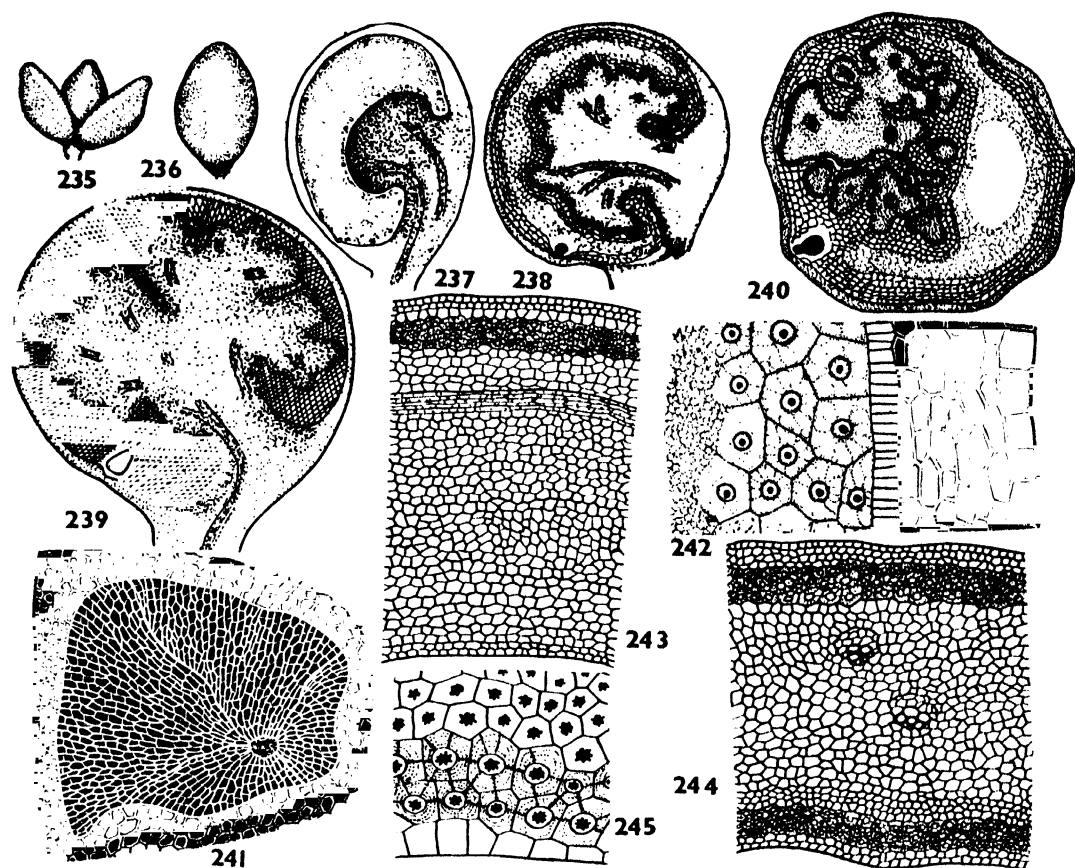
EXPLANATION OF TEXT FIGURES

TEXT-FIGURE 1. Figs. 1–31. Figs. 1–4. *Livistona rotundifolia.* Fig. 1. L. S. of young flower, ×20. Fig. 2. L. S. mature flower, ×20. Fig. 3. T. S. flower, ×30. Fig. 4. T. S. ovary, ×75. Fig. 5. T. S. flower bud of *L. chinensis,* ×15. Figs. 6–11. *Pritchardia pacifica.* Fig. 6. L. S. young flower bud, ×15. Fig. 7. L. S. pistil from the above, ×35. Fig. 8. L. S. carpel from older ovary, ×50. Fig. 9. T. S. flower bud, ×10. Figs. 10 and. 11. T. S. base and top of style, ×35. Figs. 12 and 13. *Sabal blackburniana.* Fig. 12. L. S. flower bud, ×20. Fig. 13. T. S. ovary, ×35. Fig. 14. T. S. ovary of *S. adansonis* ×20. Fig. 15. L. S. carpel of *Livistona chinensis,* ×25. Fig. 16. L. S. carpel of *Trachycarpu,* sp. ×50. Figs. 17–31. *Licuala grandis.* Fig. 17. An entire flower, ×4. Fig 18. L. S. flower, ×6. Fig. 19. Staminal tube split open, ×4. Fig. 20. Entire pistil, ×4 Fig. 21. L. S. carpel, ×20. Figs. 22–31. Series of sections of flower bud from the base towards the top; explanation in text; Figs. 26 and 27, ×20; rest ×10.

TEXT-FIGURE 2. Figs. 32–49. Organogeny of the flower in Palmae. Fig. 32. L. S. flower primordium of *Pritchardia pacifica,* ×20. Figs. 33–42. *Sabal palmetto.* Figs. 33–36. L. S. flower primordia at various stages of development, ×25. Figs. 37–41. Stages in the development of the pistil; Figs. 37 and 38, ×90; Figs. 39–41, ×50. Fig. 42. T. S. style, ×40. Figs. 43–48. *Trachycarpus* sp. Figs. 43 and 44. L. S. flower buds; Fig. 43. ×20 Fig. 44. ×30. Figs. 45 and 46 Stages in development of pistil, ×75. Fig. 47. T. S. base of pistil, ×50. Fig. 48. T. S. flower bud, ×35. Fig. 49. T. S. mature carpel of *Licuala* sp. ×50.

TEXT-FIGURE 3. Figs. 50–79. Microsporogenesis and male gametophyte in Palmae. Figs. 50–58. *Sabal palmetto.* Figs. 50–54. T. S. developing anthers showing development of wall layers and sporogenous tissue; Fig. 50, ×235; Figs. 51–54, ×475. Fig. 55. Formation of bilateral tetrad of microspores, ×475. Fig. 56. Tapetal cells and tetrahedral tetrad of microspores, ×475. Fig. 57. A young 2-celled pollen grain, ×475. Fig. 58. Tapetal cells showing cutinised inner wall, ×475. Figs. 59 and 60. *Sabal blackburniana.* Fig. 59. T. S. anther loculus, ×190. Fig. 60. T. S. anther lobe, ×90. Figs. 61–63. *S. adansoni.* Fig. 61. L. S. part of anther, ×235. Fig. 62. A microsporocyte showing cytokinesis, ×475. Fig. 63. Two-celled pollen grain, ×475. Figs. 64–69. *Pritchardia pacifica.* Fig. 64. T. S. young anther loculus showing secondary increase in sporogenous cells, ×280. Fig. 65. T. S. older anther loculus, ×135. Fig. 66. L. S. anther loculus, ×135. Fig. 67. Epidermis and fibrous endothecium, ×190. Figs. 68. and69. Pollen grains, ×475. Figs. 70 and 71. *Washingtonia.* Fig. 70. L. S. sterile anther loculus, ×110 Fig. 71. A bilateral tetrad of microspores, ×475. Figs. 72–75. Development of pollen grains in *Licuala grandis,* ×475. Figs. 76 and 77. *Trachycarpus* sp. Fig. 76. T. S. part of anther loculus; note starch in tapetal cells, ×280. Fig. 77. T. S. mature anther lobe, ×135. Figs. 78 and 79. *Livistona rotundifolia.* Fig. 78. L. S. anther loculus showing degeneration of some sporogenous cells, ×190. Fig. 79. A sterile pollen grain, ×715.

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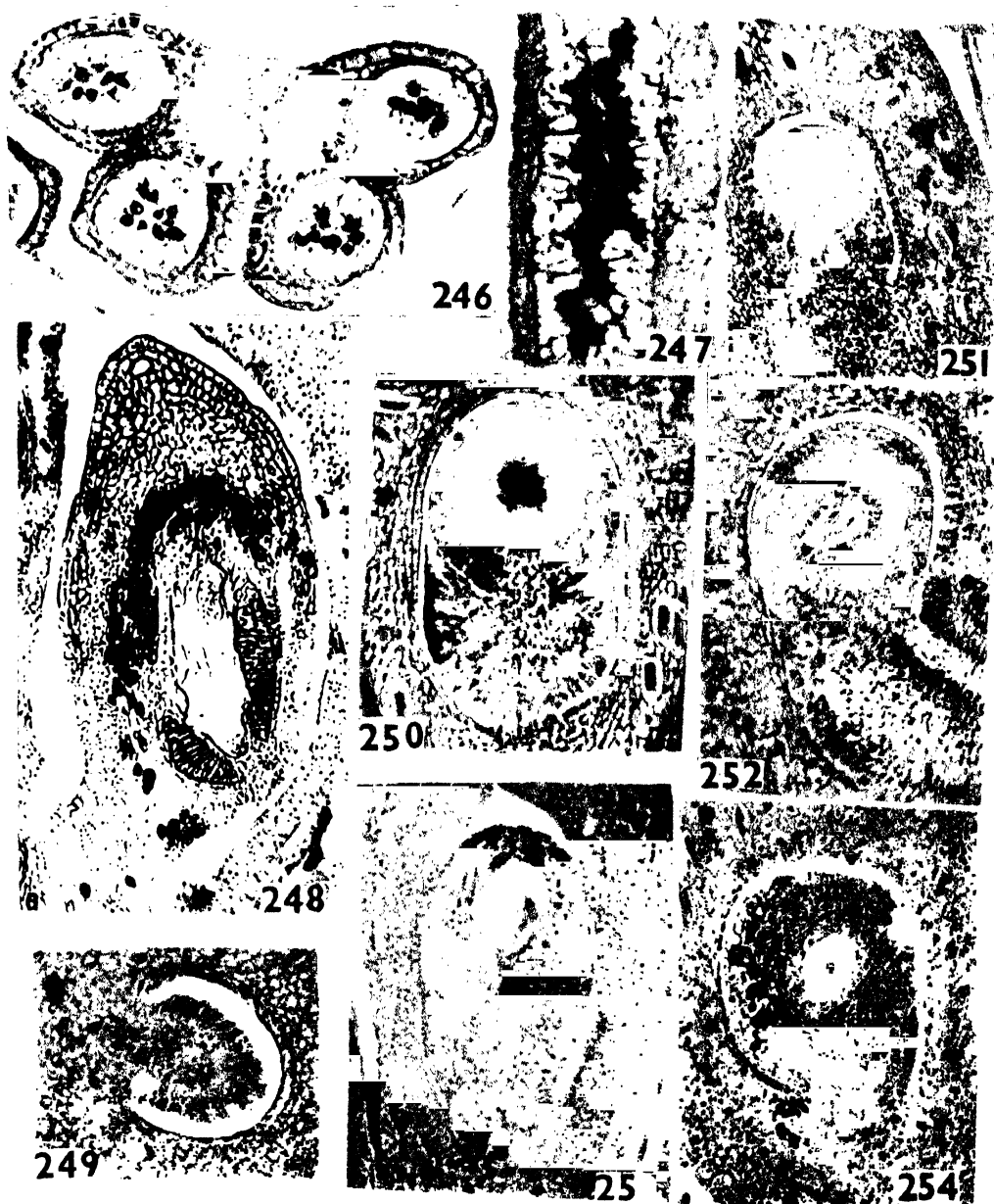
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STUDIES ON INDIAN TUNICATES. I. THE GERM CELLS IN *ECTEINASCIDIA THURSTONI* HERDMAN

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(Communicated by B. R. Seshachar, F.N.I.)

ABSTRACT

The development of the gonads in *Ecteinascidia thurstoni* Herdman (Family : Perphoridae is described. The gonads at 2mm stage of the blastozooid are seen to arise separately from their own "fundament". This is in agreement with Simkins' (1925) study on *E. turbinata*. The development of follicle cells is also studied. It appears that the 'primary follicle' is differentiated at a very early stage even when the oocyte is still a part of the germinal epithelium. The 'primary follicle' which is in the nature of a "syncytium' shows both flat and round nuclei. It is from the flat nuclei that the outer follicle cells are derived while the round nuclei give rise to the inner follicle cells. The 'Test cells' arise by mitotic divisions of the inner follicular epithelium. This study is in agreement with that of Tucker (1942) on *Styela*.

INTRODUCTION

So far as the author is aware, only two accounts of the germ cells of *Ecteinascidia* (fam : Perophoridae) exist, i.e. Lefévre (1897) and Simkins (1925). They seem to disagree in respect of the origin of the gonads. Lefévre derives the testis and the ovary from a common vesicle. This common vesicle, according to him, divides and later separates into two. From each cavity the testis and ovary arise respectively. Simkins, however, feels that they arise from their own independent "fundament".

Regarding the egg envelopes, Hüus (1937) gives a general summary of the earlier work. Some recent accounts deal with the development and function of the follicle cells—Spek (1927) in *Clavelina*, Knaben (1936) in *Corella* and Tucker (1942) in *Styela*. Earlier workers maintained that the ova and follicle cells arose from an unspecialised germinal epithelium, the inner 'test cells' differentiating from the primary follicles. The primary follicle then differentiates into an inner and outer follicular epithelium, the latter remaining continuous with the germinal epithelium, and not carried over by the ripe egg. The chorion is seen between the 'test cells' and the follicle cells. But Spek and Knaben derive the follicle cells and chorion from the amoeboid mesenchymal cells which are on the surface of the ovum. Tucker who investigated with this difference in mind confirms the earlier view (see Berrill, 1950).

In view of the paucity of information in regard to the egg envelopes in *Ecteinascidia* the present study seemed desirable.

MATERIAL AND METHODS

Ecteinascidia thurstoni Herdman was first collected during the premoonsoon period of June 1956 as part of a preliminary survey of the marine fauna of Gulf of Kutch (Gideon *et al.*, 1957). Later, in May-June 1958, many animals with

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embryos in different stages of development were collected. Financial aid for a collection trip to Okha (Western India) was generously provided by The National Institute of Sciences of India, which is gratefully acknowledged. The material was identified by Dr. R. H. Millar of the Marine Laboratory at Millport, Scotland, through the courtesy of Miss. A. M. Clark of the British Museum (Nat. Hist., London, to both of whom the author is highly thankful.

OBSERVATIONS

(1) *Compound Gonad*

The compound gonad is situated towards the left side of pharynx, above the stomach. It consists of testis and ovary enclosed in a fine, loose tissue.

Testis : It consists of follicles from which arise the vasa efferentia. The testis follicles are lined by the testicular epithelium, which is continuous with that of vas efferens. All the vasa efferentia unite mesially, generally towards the right side of the ovary to give rise to vas deferens. Neither the vasa efferentia nor the vas deferens is lined inside by cilia. The vas deferens runs along the intestine, opening into the atrium.

Ovary : The ovary appears as a bunch of grapes, situated centrally, being surrounded by the testis. It is orange in colour in the living condition. The fully formed ova are pushed towards one side of the ovary and later are conducted by a fine short oviduct to the right side of the pharynx where they undergo further development in the brood pouch. Various developmental stages from the 2–cell stage to the larva are obtained in it.

(2) *Development of the gonad*

Tadpoles about to be released from the adult revealed no indication of any germ cells. Four different stages of blastozooids were examined viz. 2 mm, 5 mm, 8 mm. and 10 mm. In the 10 mm blastozooid the gonads were clearly seen even by the unaided eye, whereas in the 5 mm. blastozooid the gonads were barely visible and in the 2 mm blastozooid the gonad was not visible to the naked eye. The 2 mm. stage, therefore, formed the starting point for the developmental studies described in this paper.

Testis : The testis arises as a group of pockets or clusters towards the left side of the atrial epithelium independently, in the region between endostyle and dorsal lamina (Fig. 1a). Each pocket is lined by the germinal epithelium. To begin with, it encloses a small cavity into which the male germ cells are shed. In the later stages these small pockets enlarge in size giving rise to the seminiferous tubules.

In the mid-region of each pocket is a small duct, the seminiferous duct or vas efferens (Fig. 1b) running towards the region of the ovary. The lining of the duct in a longitudinal section (Fig. 1c) shows elliptical nuclei, whereas in a cross section they appear round (Fig. 1d). The cavity of the duct is empty. This should be expected since the meiotic divisions start much later.

Each follicle is 50 to 60μ in thickness. In the beginning there are only a few spermatogonial cells in the follicle with much empty space. The cells stain feebly in haematoxylin. A striking feature is that all the cells in all the locules are in the same stage of development.

For the first time, at the 5 mm. stage, the nuclei begin to show some mitotic activity in that a few metaphase plates are recognised. Unfortunately, chromosome counts were not possible at this stage.

At the 8 mm. stage meiotic divisions are seen, a few sperms also. It is at this stage of development that Simkins noticed the sperms in *E. turbinata*. Finally at the 10 mm. stage the testis follicles grow larger, almost completely surrounding

the ovary. The testis follicles regress later, when the eggs in the ovary are ready to be shed.
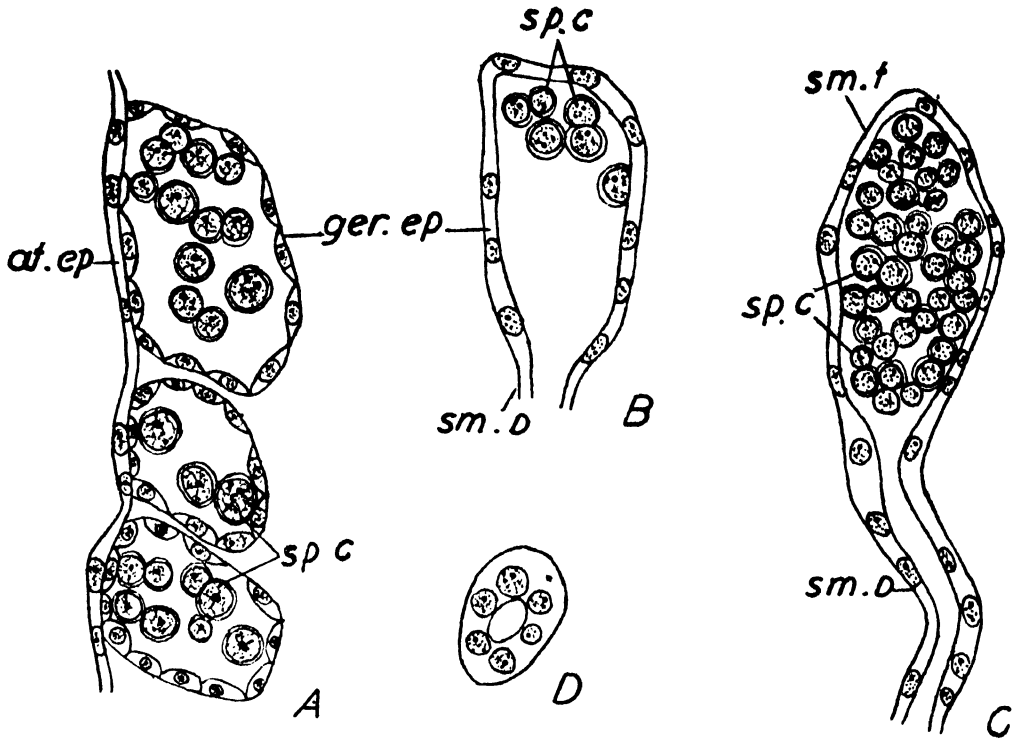
TEXT-FIG. 1

Figs. 1a to 1d. : *Development of the testis*

Fig. 1a.—Shows the testis arising as groups of pockets towards the left side of the atrial epithelium. There are a few spermatogonial cells derived from the germinal epithelium. 2 mm blastozooid. (×1200)

Fig. 1b.—Longitudinal section showing the seminiferous duct. 2 mm blastozooid (×1200)

Fig. 1c.—Longitudinal section of the seminiferous tubule and duct at a later stage (3 mm blastozooid). The cavity of the tubule is filled with spermatogonial cells. The nuclei lining the sperm duct are elliptical. (×1200)

Fig. 1d.—Cross section of the sperm duct. 3 mm blastozooid. (×2000)

Ovary : Even at 2 mm. stage, the germainal epithelium is clearly seen (Fig. 2a.) Within the germinal epithelium the oocytes begin to develop. As the oocytes grow in size, the older ones are displaced from the germinal epithelium (Fig. 2b). The oocytes appear to be in the germinal vesicle condition. The earliest oocyte resembles the spermatogonial cell. But the gradual increase in the volume of the oocyte sooner or later betrays its real identity. The fully developed oocyte ·at this stage shows an outer and an inner follicular epithelia (Fig. 3c).

In later stages the ooctyes increase in volume and only when the blastozooid is mature (10 mm.), there is seen the production of yolk in the cytoplasm. A fully developed oocyte just prior to be shed has an inner follicular epithelium and a very thin outer follicular epithelium. Internal to the inner follicular layer is seen the chorion. The 'test cells' are seen below the chorion. At this stage the

germinal vesicle breaks down. This might mark the stage at which the egg is shed from the ovary, and is conducted through a thin, membranous oviduct.
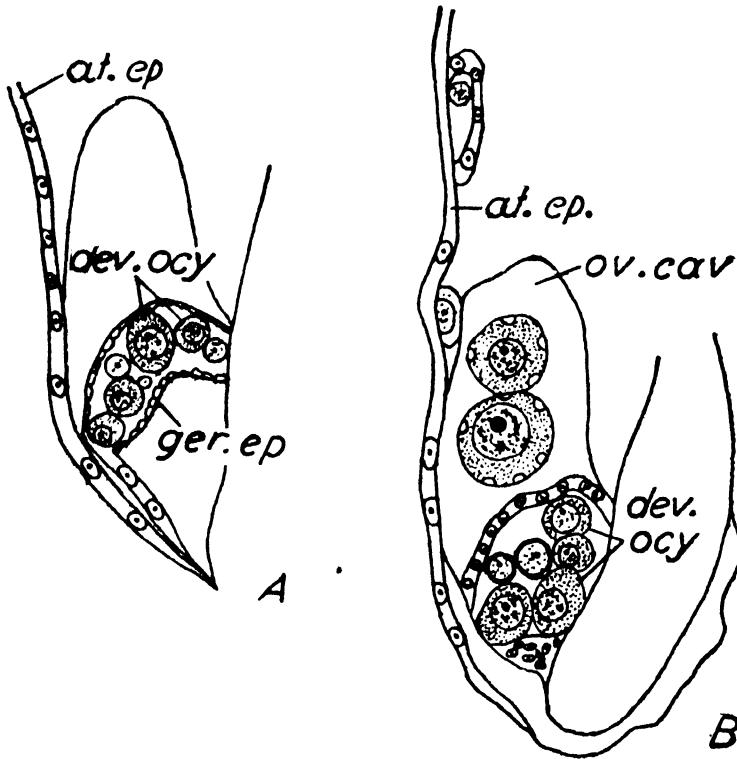
TEXT-FIG. 2

Figs. 2a to 2b. :   *Development of the ovary*

Fig. 2a.—The germinal epithelium as seen in the 2 mm blastozooid. Developing oocytes are seen.   (×600)

Fig. 2b.—The fully grown oocytes are being displaced towards one side of the germinal epithelium into the ovarian cavity. 2 mm blastozooid. (×600).

(3) *Development of egg envelopes*

It is noticed that the young oocytes are formed in the germinal epithelium while they are still part of the wall of the ovary (Fig. 2a, 3a). These young oocytes lack the follicle layers. For sometime the oocytes undergo development and later they protrude outside from the germinal epithelium, still in connection with the wall of the ovary. The oocyte is surrounded by a syncytium with flat and round nuclei (Fig. 3b). This can be regarded as the 'primary follicle'. It is from the flat nuclei that the outer follicular epithelium is derived while the inner follicle is developed from the round nuclei. This structural difference is maintained throughout. Thus the 'primary folllicle' is differentiated at a very early stage as in *Styela*. Later the outer follicle layer becomes flat and thin and many a time it may be lost during the process of section cutting, while the inner follicle layer remains intact. The follicle cells increase by mitotic divisions (Fig. 3e).

The chorion is a well defined structure in the oocyte of *Ecteinascidia*. It appears as a thick membrane in stained preparations. It lies towards the inner
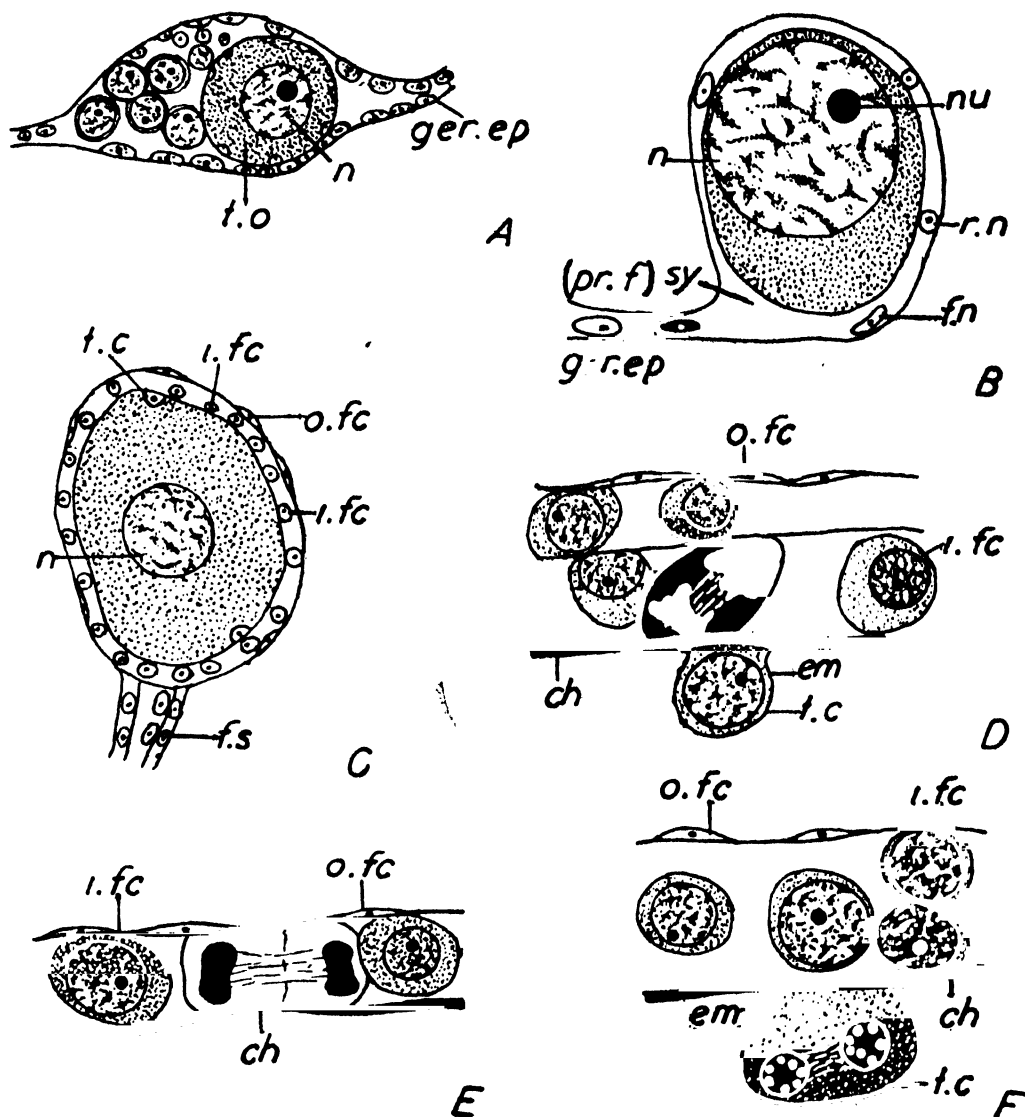
TEXT-FIG. 3.

Figs. 3a to 3f. :   *Development of egg envelopes*

Fig. 3a.—Shows the development of the oocytes from the undifferentiated germinal epithelium as tubular oocytes.   ($\times 1200$)

Fig. 3b.—The oocyte is surrounded by a syncitium, with flat and round nuclei.   The syncytium is still in connection with the germinal epithelium.   ($\times 2000$)

Fig. 3c.—A fully developed oocyte showing both outer and inner follicular epithelia.   The oocyte remains in association with the germinal epithelium through the follicular stalk.   ($\times 600$)

Fig. 3d.—Shows the origin of the 'test cell' from the inner follicle by mitotic division.   The 'test cell' is situated between the chorion and egg membrane.   ($\times 4000$)

Fig. 3e.—Shows a mitotic figure among the inner follicle cells.   ($\times 4000$)

Fig. 3f.—Shows mitotic division in the 'test cell'.   ($\times 4000$)

aspect of the inner follicular epithelium. The 'test cells' lie between the chorion and the egg membrane, as in the case of *Corella* (Knaben, 1936) and *Styela* (Tucker, 1942).

The 'test cells' arise by the mitotic divisions of the inner follicular epithelium (Fig. 3d). The increase in the number of 'test cells' and the inner follicle cells is by mitotic divisions (Fig. 3e, f).

GENERAL CONSIDERATIONS AND CONCLUSIONS

This account of the development of the gonad in *E. thurstoni* indicates that even in such an early stage as 2 mm. blastozooid the testis and ovary arise as independent entities as seen in *E. turbinata* (Simkins. 1925). But it should be pointed out here. that though the gonads appear independently of each other, nevertheless, they should at some earlier stage, have arisen from a common primordium. In this idea Lefévre (1897) is probably correct but the manner in which he assumes the differentiation into testis and ovary, needs a thorough reinvestigation.

There is a short, transparent oviduct as described in other species of perophorid ascidians by Berrill (1932) and Polugh and Jones (1939).

The egg of *E. thurstoni* resembles other viviparous ascidian eggs in the possession of a very thin outer layer. The follicle cells are never vacuolated. These conditions are associated with acquisition and maintenance of the viviparous condition (see Berrill, 1950).

As regards the origin of the follicle cells and the 'test cells' the present study is in agreement with that of Tucker's view.

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REFERENCES

Berrill, N. J. (1932). Ascidians of Bermudas. *Biol. Bull.*, **62**, 77–88.
———— —— (1950). Tunicata. Ray Society.
Gideon, (*et al.*). (1957). On the marine fauna of Gulf of Kutch : a preliminary survey. *J. Bombay nat. Hist. Soc.*, **54**, 690 706.
Hūus, J. (1937). Tunicata : Ascidiaceae. Handb. Zool. Kukenthal und Kurmboch, v, 2nd half. 545 647.
*Knaben, N. (1936). Über Entwicklung und Funktion der Testazellen bei *Corella parallelogramma* Mull. *Bergens Mus. Aarb.* 1936, 5–33.
Lefévre, G. (1897). Budding in *Ecteinascidia*. *Anat. Anz.*, **13**, 473–483.
Plough, H. H. and Jones, N. (1939). *Ecteinascidia tortugensis* species Nova. with a review of the Perophoridae (Ascidiacea) of the Tortugas. *Publ. Carneg, Instn.*, 517 47–60.
Simkins, C. S. (1925). Origin of germ cells in *Ecteinascidia*. *J. Morph.*, **39**, 295–321.
*Spek, J. (1927). Uber die Winterknospenentwicklung, Regeneration und Reduktion bei *Clavelina lepadiformis* und dei Bedeutung besonderer "Omnipotenter" Zellelemente für diese Vorgänge. *Arch. EntwMech. Org.*, Bd. cxi, 119 172.
Tucker, G. H. (1942) The histology of the gonads and the development of the egg envelopes of an Ascidian (*Styela plicata Lesueur*). *J. Morph.*, **70**, 81–108.

* not read in originals.

KEY TO THE LETTERING

at.ep.,—Atrial epithelium; *ch.*,—Chorion; *dev.ocy.*,—Developing oocytes; *e.m.*,—Egg membrane; *f.n.*,—Flat nucleus; *f.s.*,—Follicular stalk; *ger.ep.*—Germinal epithelium; *i.fc.*,—Inner follicle cell; *n.*, Nucleus; *nu.* Nucleolus; *o.fc.*, Outer follicle cell; *ov.cav.*,—Ovarian cavity; *pr.f.*,—Primary follicle; *r.n.*,—Round nucleus; *sm.d.*,—Seminiferous duct, *sm.t.*,—Seminiferous tubule; *sp.c.*,—Spermatogonial cells; *sy.*,—Syncytium; *t.c.*,—'Test cell'; *t.o.*,—Tubular oocyte;

ON THE INTERRELATIONSHIPS BETWEEN TOTAL LENGTH, STANDARD LENGTH, DEPTH AND WEIGHT OF *LATES CALCARIFER*

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(Communicated by G. P. Majumdar, F.N.I.)

ABSTRACT

In this communication an attempt has been made to analyse the interrelations between total length, standard length, depth and weight of the teleostean fish, *Lates calcarifer*. Correlation and regression analysis has been done for the original measurements and after logarithmic transformation.

INTRODUCTION

The length-weight relationship for different fishes is one of the popular subjects for fishery research of the day. After the enunciation of the cube law by Herbert Spencer in 1871 a number of investigators have worked with various kinds of fish. A more or less detailed resumé of previous results has been given by Jhingran (1952). To avoid unnecessary repetitions, the references cited in Jhingran's paper are not being mentioned here. Besides, other workers like Andrews and Lear (1956), Grainger (1953), Hewson (1955), Hourston (1952), Kennedy (1953, 1954) and Partlo (1955) have carried out valuable investigations into the length-weight relationship for fishes. In India, Bal and Joshi (1956), Karekar and Bal (1956), Pantulu (1956), Pillay (1954), Prabhu (1954), Pradhan (1956) Sarojini (1956) and others have supplied information regarding the length-weight relationships for some species of fish. Bal and Joshi (1956) have also studied the relationship between total length and standard length of Mackerel. The present investigation was undertaken with a view to studying the length-weight relationship for *Lates calcarifer*. Statistical tests establish the cube law of Spencer in this fish. Besides this, certain other points relating to the interrelationships of total length, standard length, depth and weight were also investigated ; the findings seem to be of some interest.

MATERIAL ANALYSED

The present investigation is based on measurements on 209 specimens of the food fish, *Lates calcarifer* (Bhekti), abounding in seas, back-waters and mouths of tidal rivers, from the east coast of the Persian Gulf to the Malay Archipelago and beyond (Day, 1889). These specimens were collected from local markets of Calcutta and Chandernagore (West Bengal) during the months of September to December in 1955 and during the same period in 1956. The method of selection was, strictly speaking, haphazard. The chief aim was, however, to investigate the relationships between the different measurements on the specimens. Average values of the different measurements or, more generally, the respective frequency distributions were of secondary importance. The non-randomness of the procedure of selecting specimens does not vitiate studies into the relationships so long as the ranges of values observed for the different measurements are—as in the present case—adequate ; it does, however, detract from the usefulness of the frequency distributions

or averages, measures of variability etc. presented in this paper for the different measurements.

Four different measurements were taken, viz., (i) total length of the fish, (ii) standard length, (iii) depth, and (iv) weight. Total length was the distance along the scale from the tip of the snout to the extremity of the caudal peduncle. The distance from the tip of the snout to the base of the caudal fin was taken as the standard length. Depth was measured from the base of the first spine of the anterior dorsal to the abdomen, parallel to it.

All measurements of total length, standard length and depth were taken, correct to the millimeter, on a wooden meter scale. Weights were taken, correct to 1/10th of a gram, in an ordinary physical balance.

Measurements of depth were taken for 158 specimens only. The remaining three measurements were taken for all the 209 specimens. Specimens could not be classified according to sex, since during the period of investigation maturity of the sex organs just begins.

STATISTICAL ANALYSIS : ORIGINAL VARIATES

Frequency distributions for the four measurements are given in Tables 1 and 2. It is evident from these tables that while the distributions for total length, standard length and depth are roughly symmetrical, that for weight is positively skew, which is, of course, as expected in view of the form of the relationship—discussed below—between weight on one hand and (either one or two or all of) the three remaining variates on the other.

TABLE 1

Frequency distribution of total length and of standard length of 209 specimens of Bhekti, Lates calcarifer.

Total length in cm.*	Number of fishes	Standard length in cm.*	Number of fishes
12.0—13.5	3	10—11	1
13.5—15.0	3	11—12	4
15.0—16.5	3	12—13	1
16.5—18.0	8	13—14	3
18.0—19.5	18	14—15	3
19.5—21.0	20	15—16	14
21.0—22.5	27	16—17	16
22.5—24.0	33	17—18	17
24.0—25.5	35	18—19	22
25.5—27.0	20	19—20	18
27.0—28.5	5	20—21	32
28.5—30.0	9	21—22	24
30.0—31.5	11	22—23	13
31.5—33.0	11	23—24	8
33.0—34.5	1	24—25	4
34.5—36.0	2	25—26	5
		26—27	13
		27—28	6
		28—29	3
		29—30	2
Total	**209**		**209**

* Every class-interval includes its upper limit.

TABLE 2

Frequency distribution of depth of 158 specimens and of weight of 209 specimens of Bhekti, Lates calcarifer.

Depth in cm.*	Number of fishes	Weight in gms.*	Number of fishes
3.0—3.5	2	25—50	8
3.5—4.0	10	50—75	19
4.0—4.5	11	75—100	19
4.5—5.0	16	100—125	18
5.0—5.5	23	125—150	29
5.5—6.0	38	150—175	36
6.0—6.5	35	175—200	19
6.5—7.0	10	200—225	20
7.0—7.5	2	225—250	6
7.5—8.0	4	250—275	2
8.0—8.5	4	275—300	5
8.5—9.0	1	300—325	5
9.0—9.5	2	325—350	1
		350—375	6
		375—400	5
		400—425	5
		425—450	1
		450—475	5
Total	158		209

* Every class-interval includes its upper limit.

Average values, standard deviations and ranges of the four variates are given in Table 3.† It may be mentioned that depth measurements were not available relatively frequently for the bigger fishes. This explains why means and standard deviations of the three remaining variates based on the 158 specimens for which depth was measured, are appreciably smaller than corresponding figures based on all the 209 values.

TABLE 3

Average values, standard deviations and ranges of total length, standard length, depth and weight of 209 specimens of Bhekti, Lates calcarifer.‡

Sr. No.	Variate	Unit	Average	Standard deviation	Range Minimum	Maximum	Difference
1	Total length	cm.	23.76 (22.93)	4.42 (4.20)	12.8	35.5	22.7
2	Standard length	cm.	20.26 (19.59)	3.82 (3.66)	10.9	29.8	18.9
3	Depth	cm.	(5.72)	(1.12)	(3.1)	(9.4)	(6.3)
4	Weight	gm.	176.09 (156.34)	99.38 (90.09)	28.1	470.5	442.4

‡ Figures written within brackets are based on the 158 specimens for which depth measurements were taken.

† All calculations involved in this paper were based on ungrouped data.

For want of space, only two of the scatter diagrams depicting the relationships between the variates are presented in this paper (*vide* Figure 1 and Figure 2). It was apparent from such diagrams that total length, standard length and depth are more or less linearly related among themselves, the lines of regression passing fairly close to the origin. Weight, on the other hand, bears obviously curvilinear relations with either of the three remaining variates.
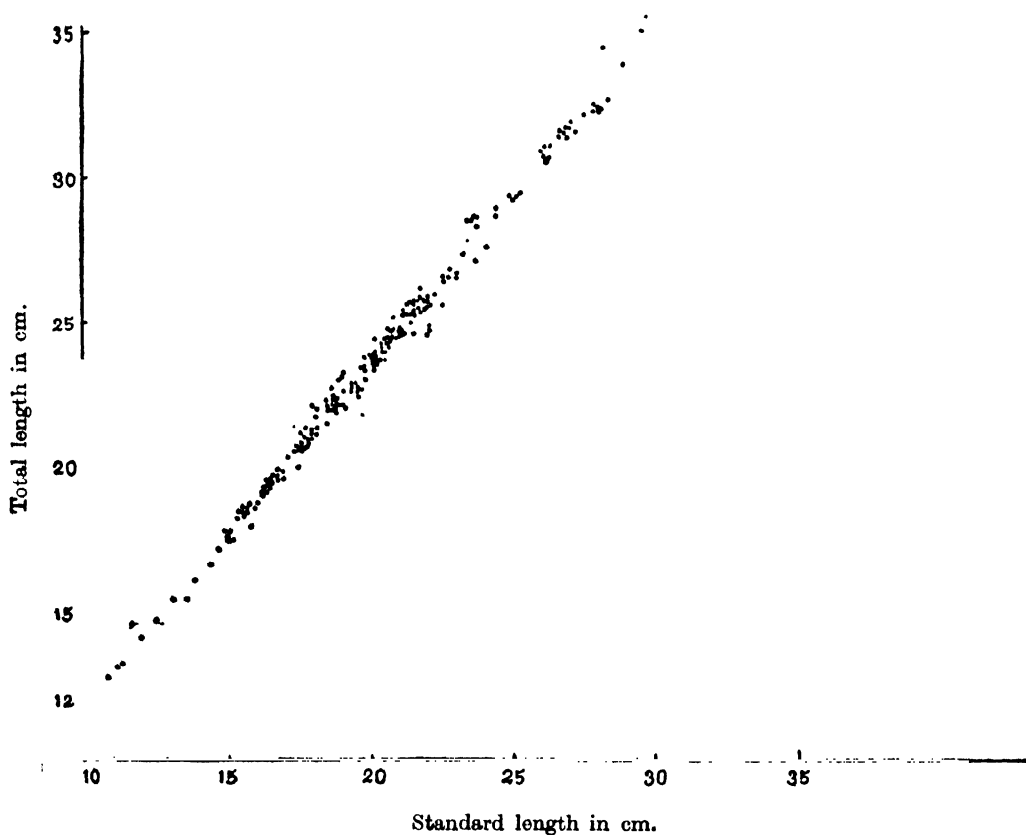
TEXT-FIG. 1.

Tests of non-linearity of regression were applied in a few cases. Specimens having the same total length, correct up to millimeter, were regarded as forming the "groups". Tables 4, 5 and 6 present the relevant analyses of variance for the regressions (in each case) on total length, of standard length, depth and weight

respectively. Contradictory to general impression, the regression of standard length on total length is significantly non-linear; it seems, however, to be of mere academic interest to fit polynomials of higher degree to this regression.
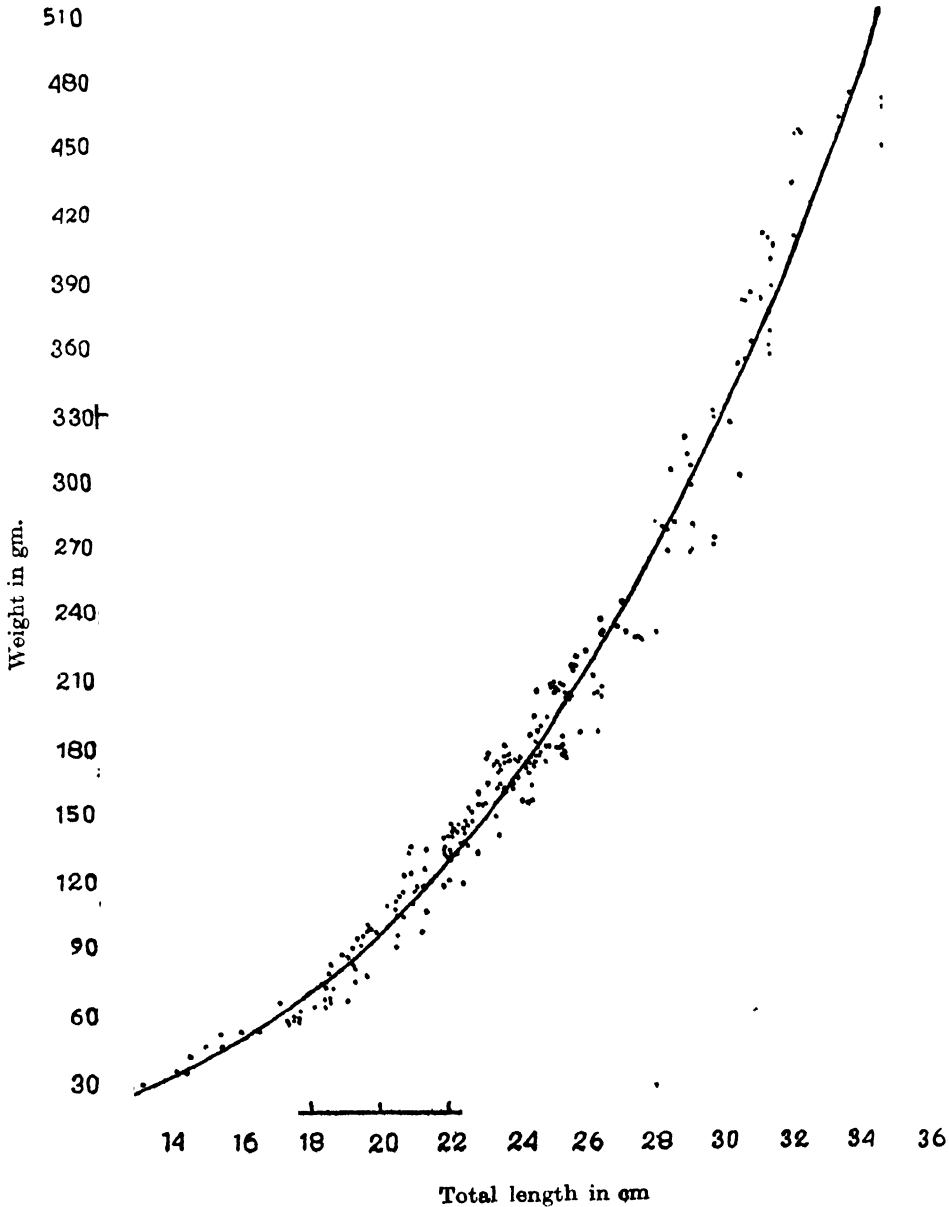
Total length in cm

TEXT-FIG. 2.

Since total length, standard length and depth are at least approximately linearly related among themselves, usual correlation and regression analysis is valid for these three variates without any transformation. Results obtained are shown

TABLE 4

Test of non-linearity of the regression of standard length on total length.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F–ratio
Linear regression	1	3010.72		
Non-linearity	110	13.38	0.1216	1.639*
Between groups	111	3024.10		
Error	97	7.20	0.07423	
Total	208	3031.30		

* The value is significant at 1 per cent level.

TABLE 5

Test of non-linearity of the regression of depth on total length.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F–ratio
Linear regression	1	178.265		
Non-linearity	88	10.539	0.11976	1.034*
Between groups	89	188.804		
Error	68	7.877	0.11584	
Total	157	196.681		

* The value is non-significant.

TABLE 6

Test of non-linearity of the regression of weight on total length.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F–ratio
Linear regression	1	1908587.89		
Non-linearity	110	132624.88	1205.68	8.98*
Between groups	111	2041212.77		
Error	97	13027.07	134.30	
Total	208	2054239.84		

* The value is significant at 0.1 per cent level.

in Tables 7 and 8 ; figures within brackets are based on the 158 specimens for whics depth was measured. Standard symbols of statistical literature have been useh (Yule and Kendall, 1950). The subscripts 1, 2 and 3, denote the three variate, viz total length, standard length and depth respectively.

TABLE 7

Ordinary, partial and multiple correlation coefficients between total length, standard length and depth.

Symbol	Value	Symbol	Value	Symbol	Value
r_{12}	0.997 (0.997)	$r_{12 \cdot 3}$	(0.969)	$R_{1 \cdot 23}$	(0.997)
r_{13}	(0.952)	$r_{13 \cdot 2}$	(0.426)	$R_{2 \cdot 13}$	(0.997)
r_{23}	(0.944)	$r_{23 \cdot 1}$	(-0.203)	$R_{3 \cdot 12}$	(0.954)

TABLE 8

Regression equations for total length (in cm.), standard length (in cm.) and depth (in cm.).

Dependent variate y	Independent variate x	Parameters of regression equation $y = a + bx$		Standard errors	
		a	b	s. e. (a)	s. e. (b)
Total length	Standard length	0.39 (0.54)	1.15 (1.14)	0.18	0.0087
Standard length	Total length	-0.20 (-0.33)	0.86 (0.87)	0.064	0.0027
Total length	Depth	(2.51)	(3.57)	(0.51)	(0.087)
Depth	Total length	(-0.10)	(0.25)	(0.16)	(0.0068)
Standard length	Depth	(1.95)	(3.08)	(0.51)	(0.087)
Depth	Standard length	(0.067)	(0.29)	(0.16)	(0.0081)

Ordinary, partial and multiple correlation coefficients between the variates after logarithmic transformation will be examined in some detail below. It is curious that coefficients in Table 7 above are very nearly the same as corresponding coefficients obtained after logarithmic transformation. To avoid repetitions, interpretations of coefficients in Table 7 are being omitted ; these will be essentially the same as those given below for the coefficients based on logarithmic variates.

Table 8 shows the standard errors of the parameters of the regressions. It will be seen that the values of a for the regressions of depth on total length or on standard length (but not *vice versa*) are not significantly different from zero. The values of a for the regressions of total length on standard length or *vice versa* are significantly different from zero. In all cases, however, in which the value of a is significant, the value is small in comparison with the range of the variates. It may be pointed out that if a is non-significant, then the relation reduces to one of proportionality.

REGRESSIONS FOR THE LOGARITHMIC VARIATES

Scatter diagrams indicating the relationships between pairs of variates after logarithmic transformation were examined, but for considerations of space only one of them has been presented here (*vide* Figure 3). It was seen that logarithms of total length, standard length and depth are also approximately linearly related among themselves. This is apparently contradictory to the findings for the corresponding regressions for original variates which are also more or less linear. The small values of '*a*' in Table 8 seem to be responsible for this somewhat anomalous result. It was also evident from the diagrams that the logarithm of weight varies linearly with either of the three remaining logarithmic variates. This latter is in confirmation of the well-known Spencer's cube law, or more properly speaking its generalisation, i.e. the allometric growth formula of Huxley.
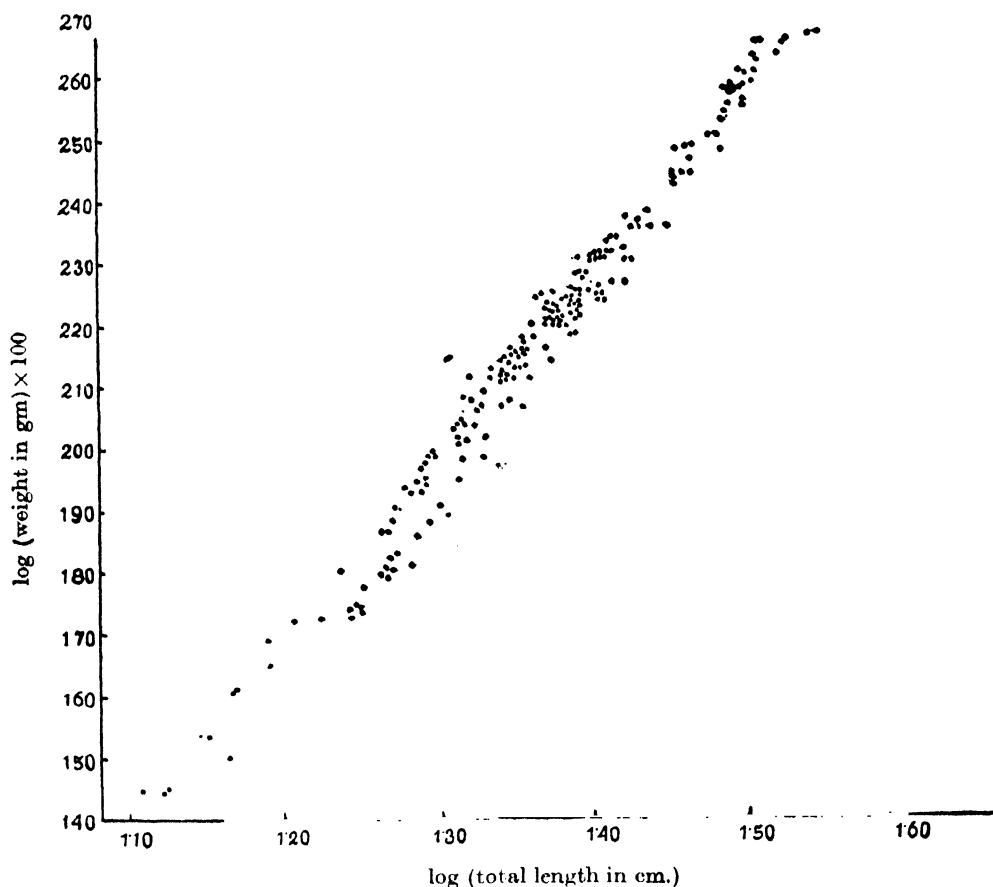
TEXT-FIG. 3.

By way of example, the analysis of variance for testing linearity of the regression, of (logarithmic) depth on (logarithmic) total length has been presented in Table 9. As before, specimens having the same total length, correct up to millimetre formed the "groups". The significant result in Table 9 makes it plausible that the fits of some of the regressions in Table 10 can be improved by adding higher degree terms ; the linear regressions are, however, quite sufficient for most purposes. Table

10 presents some linear regression equations (fitted by the method of least squares) for the logarithmic variates.

TABLE 9

Test of non-linearity of the regression of logarithm of depth on logarithm of total length.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F-ratio
Linear regression	1	1.040302		
Non-linearity	88	0.075357	0.00085633	1.449*
Between groups	89	1.115659		
Error	68	0.040191	0.00059104	
Total	157	1.155850		

* The value is just significant at the 5 per cent level.

TABLE 10

Regression equations for the logarithms of total length in cm. (x_1), standard length in cm. (x_2), depth in cm. (x_3) and weight in gm. (x_4).

Sr. No.	Dependent variate	Independent variate or variates	Regression equation	s. e. (b)
1	$\log x_4$	$\log x_1$	$\log x_4 = -1.980 + 3.038 \log x_1$	0.029
2	$\log x_4$	$\log x_2$	$\log x_4 = -1.704 + 2.989 \log x_2$	0.035
3	$\log x_4$	$\log x_3$	$\log x_4 = 0.032 + 2.798 \log x_3$	0.056
4	$\log x_1$	$\log x_2$	$\log x_1 = 0.089 + 0.985 \log x_2$	0.005
5	$\log x_2$	$\log x_1$	$\log x_2 = -0.083 + 1.010 \log x_1$	0.005
6	$\log x_1$	$\log x_3$	$\log x_1 = 0.683 + 0.894 \log x_3$	0.024
7	$\log x_3$	$\log x_1$	$\log x_3 = -0.613 + 1.006 \log x_1$	0.027
8	$\log x_2$	$\log x_3$	$\log x_2 = 0.607 + 0.905 \log x_3$	0.064
9	$\log x_3$	$\log x_2$	$\log x_3 = -0.509 + 0.979 \log x_2$	0.028
10	$\log x_4$	$\log x_1$ $\log x_3$	$\log x_4 = -1.387 + 2.077 \log x_1$ $+ 0.941 \log x_3$	
11	$\log x_4$	$\log x_2$ $\log x_3$	$\log x_4 = -1.110 + 1.866 \log x_2$ $+ 1.110 \log x_3$	

Tests of significance were applied to examine whether the regression coefficients in line numbers 1, 2 and 3 of Table 10 are different from 3. The critical ratios which are normally distributed in virtue of the large sample sizes, are 1·30,–0·32, and –3.60 respectively. The non-significance in the first case implies that Spencer's law holds good for *Lates calcarifer*.* In a similar manner it is found that regression coefficients in lines 4 and 5 are significantly different from 1, which shows that the relation between total length and standard length is not one jof proportionality. For that of total length on depth the deviation is still more conspicuous ; but for standard length and depth, a relation of proportionality may be assumed. Detailed examination will show the correspondence between significance of values of a in Table 8 and of significance of deviations of values of b from 1 in Table 10.

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* It is possible that some of the cases of 'failure' of Spencer's law reported in the literature would turn out to be otherwise on an application of tests of significance.

Table 11 gives expected or standard values of weight (in gms.) for different values of total length, standard length or depth. These values are based on regressions of Table 10.

TABLE 11

Standard values of weight corresponding to different values of total length, standard length or depth of Bhekti, Lates calcarifer.

Total length (cm.)	Weight (gms.)	Standard length (cm.)	Weight (gms.)	Depth (cm.)	Weight (gms.)
				3	23.3
10	11.4	10	19.3		
				4	52.1
15	39.2	15	64.2		
				5	97.2
20	94.0	20	152.8		
				6	161.9
25	185.2	25	297.7		
				7	249.3
30	322.2	30	513.3		
				8	362.2
35	514.7				
				9	503.6

CORRELATIONS AMONG THE LOGARITHMIC VARIATES

The matrix of correlation coefficients between the logarithmic variates is given in Table 12. Figures within brackets represent correlation coefficients based on the 158 sets of values for which depth measurements were available.

Tables 13, 14 and 15 give respectively the partial correlation coefficients of the first order, the partial correlation coefficients of the second order, and the multiple correlation coefficients. Subscripts 1, 2, 3 and 4 denote the four variates i.e. respectively, logarithms of total length, standard length, depth and weight. In Table 13 a few figures are based on 209 sets of values and the remaining ones, given within brackets, on 158 sets of values only.

TABLE 12

Matrix of correlation coefficients between logarithms of total length, standard length, depth and weight of 209 specimens of Bhekti, Lates calcarifer.

Logarithm of	Logarithm of			
	Total length	Standard length	Depth	Weight
Total length	1	0.997 (0.998)	0.948	0.991 (0.988)
Standard length		1	0.941	0.986 (0.984)
Depth			1	(0.970)
Weight				1

TABLE 13

Partial correlation coefficients of the first order for the logarithmic variates.

Symbol	Value	Symbol	Value	Symbol	Value
$r_{12 \cdot 3}$	(0.979)	$r_{13 \cdot 4}$	(−0.261)	$r_{23 \cdot 4}$	(−0.318)
$r_{12 \cdot 4}$	0.894	$r_{14 \cdot 2}$	0.569	$r_{24 \cdot 1}$	−0.136
	(0.920)		(0.512)		(−0.128)
$r_{13 \cdot 2}$	(0.423)	$r_{14 \cdot 3}$	(0.883)	$r_{24 \cdot 3}$	(0.867)
		$r_{23 \cdot 1}$	(−0.237)	$r_{34 \cdot 1}$	(0.671)
				$r_{34 \cdot 2}$	(0.734)

TABLE 14

Partial correlation coefficients of the second order for the logarithmic variates.

Symbol	Value	Symbol	Value
$r_{12 \cdot 34}$	0.914	$r_{23 \cdot 14}$	−0.205
$r_{13 \cdot 24}$	0.084	$r_{24 \cdot 13}$	0.029
$r_{14 \cdot 23}$	0.332	$r_{34 \cdot 12}$	0.665

TABLE 15

Multiple correlation coefficients for the logarithmic variates.

Symbol	Value	Symbol	Value
$R_{1 \cdot 234}$	0.998	$R_{3 \cdot 124}$	0.973
$R_{2 \cdot 134}$	0.998	$R_{4 \cdot 123}$	0.993

The following are the salient features of the above-mentioned tables :

(a) All the ordinary correlation coefficients are very high. The minimum figure is 0.941, which is the correlation coefficient between (the logarithm of) standard length and (that of) depth. The mutliple correlation coefficients are also very close to 1, 0.973 (for the logarithm of depth) being the lowest value. Any of the four variates can thus be predicted from the others with great accuracy. (Logarithmic depth is the least correlated of the four variates. The other three are more highly correlated with the rest.) It is, however, seen from a comparison of the ordinary and multiple correlation coefficients that although many of the partial correlation coefficients are significant, there is very little gain in accuracy in predicting the logarithm of weight or of depth and still less in predicting (the logarithms of) total length or standard length from the other logarithmic variates) with an increase in the number of predictor variates beyond one.

(b) The correlation coefficient between total length and standard length (both logarithmic) is very nearly unity. All the partial correlation coefficients (viz.

3

$r_{12\cdot3}$, $r_{12\cdot4}$, $r_{12\cdot34}$) between these two variates are also very high. Moreover, in all cases an ordinary or partial correlation coefficient *involving one of these two variates but not the other* is seen to be more or less unchanged if one of these variates is substituted for the other[1]. (Compare, for example, $r_{14\cdot3}$ and $r_{24\cdot3}$ or $r_{34\cdot1}$ and $r_{34\cdot2}$.) These facts render either of the two measurements an almost perfect indicator of the other[2]. This is somewhat contradictory to the statement of Jhingran (1952). Jhingran clearly recognised the superiority of standard length, but stated that its measurement was more difficult. He used furcal length instead of total length fearing that the latter would be affected by 'wear and tear'. (Furcal length, of course, does not exist for *Lates calcarifer*.) The present study seems to indicate that the effect of 'wear and tear' on total length of *L. calcarifer* is not so serious, for inspite of it, total length is an extremely good indicator of standard length. It is suggested that in most studies in which standard length has been used, the easier-to-measure total length would have sufficed for all practical purposes.

(c) The partial correlation coefficients of the first order provide interesting study although quite a few are difficult to interpret. For some of the coefficients of the second order, the physical significance is still more obscure. The partial correlation coefficients with 1 as a primary suffix and 2 as a secondary suffix, or *vice versa*, are particularly in point. All these coefficients are highly significant excepting $r_{24\cdot1}$ which is nearly significant at 5 per cent level, and $r_{13\cdot24}$ and $r_{24\cdot13}$ which are non-significant.

(d) High and positive values of $r_{12\cdot3}$, $r_{12\cdot4}$ and $r_{12\cdot34}$ are, as stated earlier, quite expected. These indicate that even when weight and/or depth is held fixed, standard length and total length are closely related. The values of $r_{14\cdot3}$ and $r_{24\cdot3}$, it may be noted, are higher than those of $r_{34\cdot1}$ or $r_{34\cdot2}$ or, what comes to nearly the same thing, $r_{34\cdot12}$. This shows that weight is more heavily dependent on total length or standard length than on depth, a fact which is not so obvious from the ordinary correlation coefficients themselves.

(e) As regards the remaining coefficients in Tables 13 and 14, only the negative values of $r_{13\cdot4}$ and $r_{23\cdot4}$ are intelligible. These indicate that if weight is to remain fixed, standard length or total length must decrease as depth is increased. All the remaining coefficients have the subscript 1 as a primary suffix and 2 as a secondary suffix or *vice versa*, which leads to difficulties in interpretation. It appears from the values of $r_{13\cdot2}$ and $r_{14\cdot2}$ that for a given standard length, an increase in total length is associated with increasing weight, which is expected, and with increasing breadth. As against this, the values of $r_{23\cdot1}$ and $r_{24\cdot1}$ indicate that for a fixed total length, increasing standard length is associated with decreasing weight or breadth. A somewhat similar contrast may be noticed between the values of $r_{14\cdot23}$ and $r_{23\cdot14}$.

Remarks

The significant traces of non-linearity of some of the regressions between total length, standard length and depth have been ignored in this paper ; it does not seem important to estimate the higher degree terms of these regressions. For identical reasons, the slight non-linearity of some of the regressions between logarithmic variates have not been analysed. It is apprehended that in studies with such high correlation coefficients, traces of non-linearity in some of the regressions may distort the partial correlation coefficients.

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[1] It will be seen, that, for example, $r_{14\cdot2}$ and $r_{24\cdot1}$ are not of the same order of magnitude, which explains the clause italicised.

[2] This is, of course, quite natural since standard length is the major component of total length. Besides, the remaining component of total length (which is in fact the length of the caudal fin) is also correlated with standard length. For the 209 specimens taken together, this correlation coefficient was found to be 0.848.

A few points emerging out of this study seem to be of some theoretical interest, viz. the interrelationships between the linearity or otherwise of the regressions for original and transformed variates, of the significance of the value of '*a*' for the former and of the deviation of '*b*' from 1 for the latter etc.

SUMMARY

Total length, standard length and depth are more or less linearly related among themselves, both before and after logarithmic transformation ; in some cases the relation is one of approximate proportionality. Weight, on the other hand, bears curvilinear relationships to these variates ; these, however, become roughly linear after logarithmic transformation. The relationship of total length and weight is not significantly different from Spencer's cubic law.

Other interesting conclusions emerge out of the detailed regression and correlation study. One of some practical importance is that for most purposes total length may be used instead of standard length.

REFERENCES

Andrews, C. W. and Lear, E. (1956). The biology of arctic char (*Salvelinus alpinus* L.) in northern Labrador. *J. Fish. Res. Bd Can.*, **13**, (6), 843–860.

Bal, D. V. and Joshi, M. S. (1956). Studies on the biology of *Coilia dussumieri* (Cuv. and Val.) *Indian J. Fish.*, **3**, (1), 91–100.

Day, F. (1889). The Fauna of British India : Fishes, Vol. I. Taylor and Francis, London.

Grainger, E. M. (1953). On the age, growth, migration, reproductive potential and feeding habits of the arctic char (*Salvelinus alpinus*) of Frobisher Bay, Baffin Island, *J. Fish. Res. Bd Can.*, **10**, (6), 326–370.

Hewson, L. C. (1955). Age, maturity, spawning and food of burbot (*Lota lota*) in Lake Winnipeg. *Ibid*, **12**, (6), 930–940.

Hourston, A. S. (1952). The food and growth of the maskinonge (Eson masquinongy Mitchill) in Canadian Waters. *Ibid*, **8**, (5), 347–368.

Jhingran, V. G. (1952). General length weight relationship of three Major carps of India, *Proc. nat. Inst. Sci. India*, **18**, (5), 449–460.

Karekar, P. S. and Bal, D. V. (1956). Interrelationships between standard length, body weight, gonad-length and gonad-weight of *Polydactylus indicus*. *Indian Sci. Cong. Abstract.*, 305.

Kennedy, W. A. (1953). Growth, maturity, fecundity and mortality in the relatively unexploited white fish, *Coregonus clupeaformis*, of Great Slave Lake. *J. Fish. Res. Bd Can.*, **10**, (7), 413–441.

——— (1954). Growth, maturity and mortality in the relatively unexploited lake trout, *Cristivomer namaycush*, of Great Slave Lake. *Ibid*, **11**, (6), 827–852.

Pantulu, V. R. (1956). Studies on the biology of the Indian fresh-water eel., *Anguilla bengalensis* gray. *Proc. nat. Inst. Sci. India*, **22**, (5), 259–280.

Partlo, J. M. (1955). Distribution, age and growth of Eastern Pacific Albacore (*Thunnus alalunga* Gmelin). *J. Fish. Res. Bd Can.*, **12**, (1), 35–60.

Pillay, T. V. R. (1954). The biology of the Grey Mullet, *Mugil tade* Forskal, with notes on its fishery in Bengal. *Proc. nat. Inst. Sci. of India*, **20**, (2), 187–217.

Prabhu, M. S. (1954). The perch-fishery by special traps in the area around Mandapam in the Gulf of Mannar and Palk Bay. *Indian J. Fish.*, **1**, (1) & (2), 94–129.

Pradhan, L. B. (1956). Mackerel fishery of Karwar. *Ibid.*, **3**, (1), 141–185.

Sarojini, K. K. (1956). Biology and fisheris of the Grey Mullets of Bengal. I. Biology of *Mugil Parsia* Hamilton with notes on its fishery in Bengal. *Ibid*, **4**, (1), 160–207

Yule, G. U. and Kendall, M. G. (1950). Introduction to the Theory of Statistics. 14th Edition, Charles Griffin & Co., London.

MORPHOLOGY OF THE ALIMENTARY CANAL OF THE LARVA OF *LEUCINODES ORBONALIS* GUEN. (LEPIDOPTERA, PYRAUSTIDAE)

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Abstract

The foregut is divisible into pharynx, oesophagus, crop, oesophageal valve and the anterior interstitial ring. A blood sinus is distinctly observed in the oesophageal valve but as it is without a chitinous annular ring and the restraining cords it is not considered to be capable of acting as a press for the formation of peritrophic membrane.

The midgut epithelium contains interstitial cells, columnar cells and goblet cells. The interstitial cells regenerate the columnar and goblet cells which form the regular epithelium of the midgut. The globular protrusions arising from the columnar cells are regarded to be cell disintegration products and not the secretory vesicles. The peritrophic membrane is secreted by midgut cells of the anterior region and is not reinforced by the secretions from the cells of midgut along the whole of its length.

The hindgut is divisible into ileum, anterior sphincter region, colon, a feebly developed posterior sphincter region and rectum.

Introduction

The alimentary canal of the lepidopterous larvae has certain interesting features which have engaged the attention of several workers. But there is no unanimity among them over various issues. Among those are the functional and morphological relationships of different kinds of the midgut epithelial cells, the process of secretion, the mode of formation, occurence and function of peritrophic membrane and a blood sinus in the oesophageal invagination. A detailed morphological study of the alimentary canal of the larva of *L. orbonalis* was taken up to investigate these features and the observations made are recorded in the present paper.

Material and Technique

The larvae were collected from the infected brinjals and were reared in the laboratory. The instars were identified on the basis of head widths which were found to be 0.20 mm., 0.42 mm., 0.72 mm. and 1.36 mm. in first, second, third and fourth instars respectively. The various parts of the alimentary canal were sectioned at 6–8 micra thickness by usual microtomical methods and the sections were stained by haematoxyline and eosine.

Observations

The alimentary canal is almost a straight tube, nearly as long as the larva itself (Fig. 1) and having a wide lumen. As usual the gut is differentiated into the foregut, midgut and hindgut of which the foregut and the hindgut are fairly small while the midgut occupies almost the whole of the abdomen. The exact dimensions of these divisions vary from individual to individual depending upon the stage of development, quantity of food material in the lumen and the peristaltic phase in which the gut is fixed.

1. The Foregut

The foregut starts from the level of the mouth and extends backwards upto the metathoracic segment where it joins the midgut. Its wall is highly elastic and translucent. It fills the entire thoracic cavity when full of food but when empty it is much reduced in diameter with several infoldings in the wall. The following histological layers can be distinguished : (1) an enveloping membrane of connective tissue, (2) two layers of muscle fibres, (3) a single layer of epithelial cells resting on a basement membrane, and (4) a thin layer of chitinous intima lining the epithelium continuous with the chitin of the external bodywall.

The foregut can be differentiated into five regions, viz., pharynx, oesophagus, crop, oesophageal valve and anterior interstitial ring (Borla's generative region or Hufnagel's anterior imaginal ring). The oesophageal valve is the conspicuous double layered fold which the oesophagus forms at its junction with the midgut (Fig. 8). Between the posterior fold of this valve and the midgut epithelium there is a ring of very small rapidly proliferating cells called the anterior interstitial ring. Immediately anterior to the oesophageal fold the foregut dilates to form the crop which tapers anteriorly into a narrow tube of which the posterior half is the oesophagus and the anterior half the pharynx which continues into the mouth cavity.

(1) *Pharynx*

The pharynx (Fig. 1) is confined to the head region. Its posterior limit is defined by the attachment of posterior dilator muscles to its wall. In longitudinal sections (Fig. 4) this limit can also be detected by the sudden thickening of the intima at the commencement of the oesophagus. Throughout its length the epithelial lining is thrown into a number of folds projecting into the lumen of the gut (Fig. 2). The epithelial layer seems to form a thin syncitium, the margins of the individual cells are indistinguishable from each other. The presence of prominent nuclei in the epithelium at certain places causes the surface to bulge out. The chitinous intima lining the epithelial layer internally is very prominent but is not provided with bristles as observed by Henson (1931) in *Vanessa*.
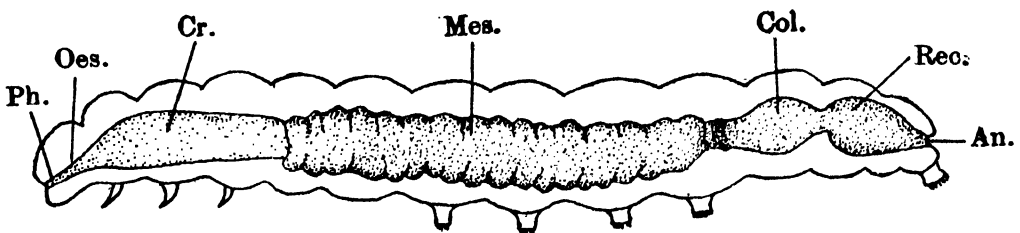
Fig. 1

Alimentary canal of the larva showing gross anatomy (diagrammatic).
An., Anus; *Col.*, Colon; *Cr.*, Crop; *Mes.*, Mesenteron; *Oes.*, Oesophagus; *Ph.*, Pharynx; *Rec.*, Rectum.

(2) *Oesophagus*

It extends from the posterior end of the pharynx beyond the level of the attachment of the dilator muscles and continues beyond the level of dorsal margin of the head capsule (Fig. 1). Its posterior limit is marked by the absence of longitudinal foldings of the epithelium and by the increased width of the lumen

(Fig. 5). The epithelium is similar to that of the pharynx but the chitinous intima of the oesophagus is thicker and bears backwardly directed spines (Fig. 3). The
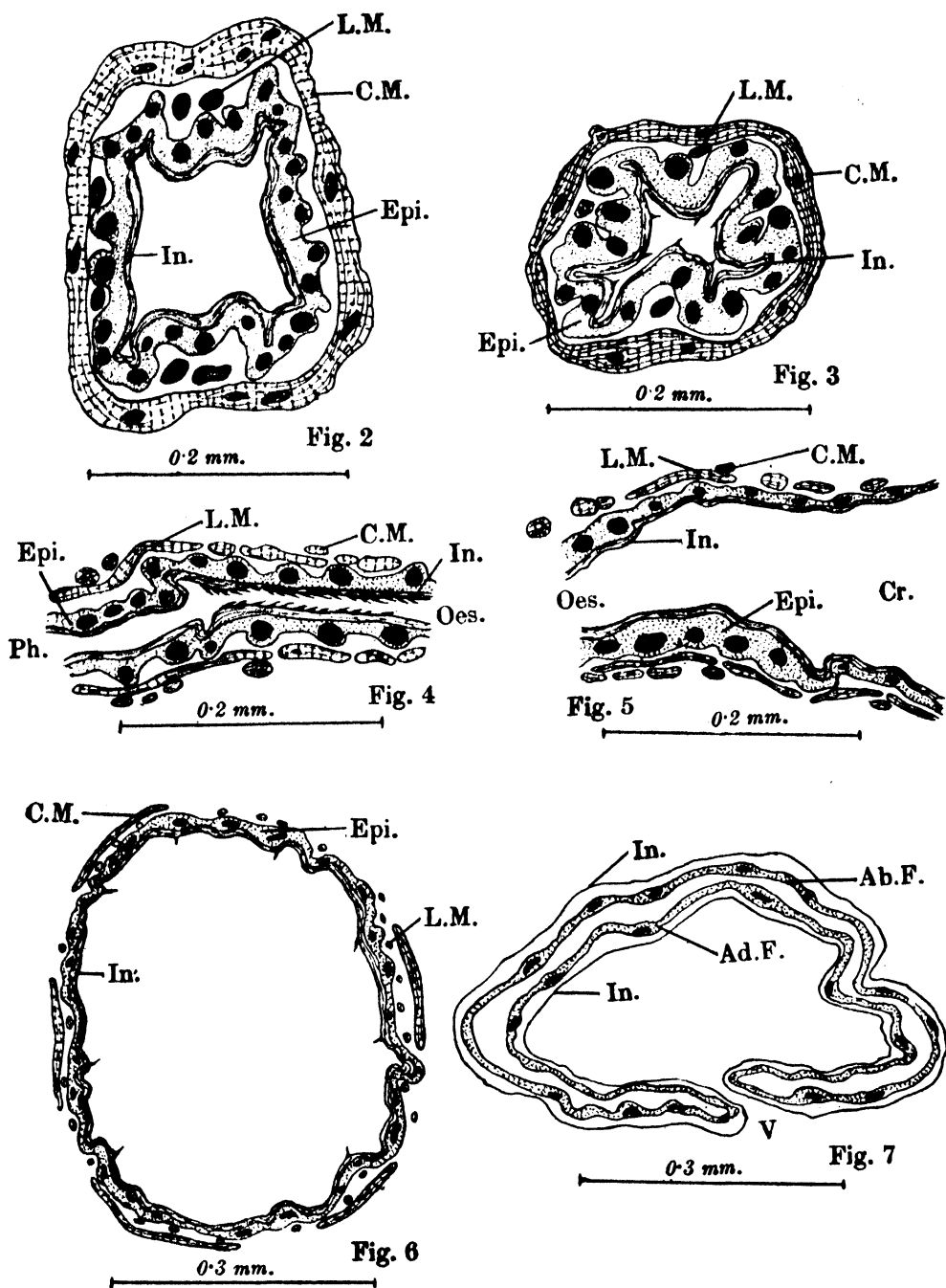
Fig. 2, T.S. Pharynx; Fig. 3, T.S. oesophagus; Fig. 4, L.S. through pharynx and oesophagus. Fig. 5, L.S. through oesophagus and crop; Fig. 6, T.S. crop; Fig. T.S. oesophageal valve. *Ab.F.*, Abaxial fold; *Ad.F.*, Adaxil fold; *C.M.*, Circular muscles; *Cr.*, Crop; *Epi.*, Epithelium; *In.*, Intima; *L.M.*, Longitudianal muscles; *Oes.*, Oesophagus; *Ph.*, Pharynx; *V.*, Valve.

epithelium is thrown into six longitudinal folds which are all equally well developed. Both longitudinal and circular muscles are well developed but the former are discontinuous and form several longitudinal bands separate from one another.

(3) *Crop*

It is the largest region of the foregut and is in the form of a pear-shaped sac situated in the pro- and mesothoracic segments (Fig. 1). Its thin epithelial wall is formed of large flattened cells with their margins merged with each other (Fig. 6). The nuclei are quite distinct but are smaller than those of the pharynx and the oesophagus. The chitinous intima is very thin and possesses a few fine bristles. The epithelium is not produced into folds which are quite conspicuous in the pharynx and the oesophagus. Like *Vanessa* (Henson, 1931), the midgut comes much further forward on the ventral side than on the dorsal side. Hence the crop is attached obliquely to the anterior end of the midgut.

(4) *Oesophageal Valve*

In this region the oesophagus is invaginated into the lumen of the midgut (Fig. 7) and in a L.S. (Fig. 8) it appears to hang down in the form of a double walled pocket-like fold. A blood sinus fills the fold of the valve. The structure of the wall of the fold resembles that of the oesophagus proper. The epithelial layer and the chitinous intima investing it from within continue in the walls of the fold. The intima is smooth and is neither provided with spines nor is it thickened to form a conspicuous annular ring on the posterior edge of the invagination. The sinus contains blood which is seen in the form of reticular mass in the sections. Because of the absence of any restraining cords or muscles the oesophageal valve in this insect seems inefficient to serve as a press for the peritrophic membrane.

(5) *Anterior Interstitial Ring*

In between the abaxial fold of the oesophageal valve and the midgut epithelium is a small part of the gut wall (Fig. 8) showing a layer of small nuclei. This forms the interstitial region. The walls limiting the boundaries of the cells in this region are not perceptible. There is a chitinous intima lining the layer from within, which is continuous with the layer of intima over the oesophageal valve and the foregut. It is thus clear that this region forms a part of the stomodaeum. A few cells at the extreme anterior end of the migdut also can not be easily distinguished in shape and form from the cells of the interstitial region.

2. THE MIDGUT

It extends from the mesothoracic segment to the sixth or the middle of the seventh abdominal segment (Fig. 1). Unlike the foregut the midgut is an undifferentiated tube of uniform diameter throughout its length. It is formed of the following layers: (1) An enveloping membrane of connective tissues, (2) two layers of muscle fibres, (3) a single layer of midgut epithelial cells resting on a basement membrane, and (4) the peritrophic membrane.

At both the anterior and the posterior limits of the midgut the epithelial cells become gradually smaller in size (Figs. 8 and 13) till they reach the normal dimensions of the cells of the anterior and the posterior interstitial rings respectively. It is therefore not possible to determine the anterior and posterior limits of the midgut epithelium. It has been suggested that these transitory cells have arisen from the cells of the interstitial rings. Despite the transitory cells, the anterior

limit of the midgut is indicated clearly by the abrupt discontinuance of the thick musculature of the foregut.

The musculature of the midgut has essentially the same character throughout its length. It consists of an outer layer of longitudinal muscle fibres and an inner layer of circular fibres (Fig. 12). The longitudinal muscle fibres are quite numerous and the distance between them varies with the age and also the degree of relaxation or distention of the gut. On the whole, the muscular coat is quite thin as compared to that of the fore and the hindgut.

(1) *Midgut Epithelium*

The single layer of midgut epithelium (Fig. 12) which rests on a conspicuous basement membrane consists of three types of cells viz., (a) the ordinary columnar or cylindrical cells constituting the majority, (b) the calciform or goblet cells lying scattered in between the columnar cells, and (3) a few interstitial or regenerative cells which lie scattered singly or in groups at the basses of the former two types.

Interstitial Cells (Fig. 12). Two types of digestive cells originate from the regenerative or interstitial cells. These cells either occur in groups called nidi or are scattered singly all over the base of midgut epithelium. They are generally very small but variable in size, roughly triangular, with prominent nuclei and often with indistinct cell walls. They are covered over by tall columnar and goblet cells.

Columnar Cells (Fig. 12). The large columnar cells outnumber other types of cells in the midgut epithelium. Their dimension depends on the stage of development and on the degree of the contraction and relaxation of the gut wall. These cells possess an internal 'striated border' of variable height which is highest in the anterior part of the midgut where the cells are taller than the posterior part. The striated border may be regarded to increase the cell surface for these activities. It may as well serve to discharge the secretion more uniformly. These cells have distinct lateral walls and their basses rest on a thin basement membrane. The cytoplasm is clear and finely granular. The nucleus which occupies a central place is prominent and is somewhat ovoidal in shape. In the distal ends of many full sized columnar cells are observed some globular or vesicular protrusions which appear arising from the lumen ends of the cells and are particularly abundant in the older larvae. Such protrusions are never observed in smaller or newly formed columnar cells. These globules are sometimes clear but are often partially or completely filled with a granular substance which stains much like the cytoplasm of the cell. The vesicles are often regarded as secretory globules but the evidence here clearly indicates that they are nothing but cell disintegration products and are not secretory in function.

Goblet Cells (Fig. 12). These cells have more or less the same form as the columnar cells but the upper half of each is occupied by a typical globlet due to which they are usually expanded in their middle part. Occasionally the goblet opens at the distal end of the cells. The cavities of the goblets appear clear and their lining is much like the striated border of the columnar cells. Their morphological nature strongly suggests that they may be the invaginations of the distal cell surfaces of the ordinary columnar cells. The size of the goblet is very variable in different cells and depends on the stage of their development. Due to the presence of the goblet the cytoplasm is accumulated at the basal region of the cell which also accommodates the nucleus.

(2) *Activities of the Midgut Epithelium*

The midgut epithelium is concerned with secretion and absorption and shows degeneration and regeneration of cells during or following secretion and the disintegration of the epithelium at the commencement of the metamorphosis. Since

these phenomenon are repeated uniformly in each instar, the observations were taken in the various developmental stages of the third instar larvae only.

In the very early third instar larvae (Fig. 9) i.e. immediately after the second moulting the interstitial cells are large in number and situated separately or in groups. All stages of development from small interstitial cells to the normal sized columnar cells are observable. Some of the interstitial cells are as tall as
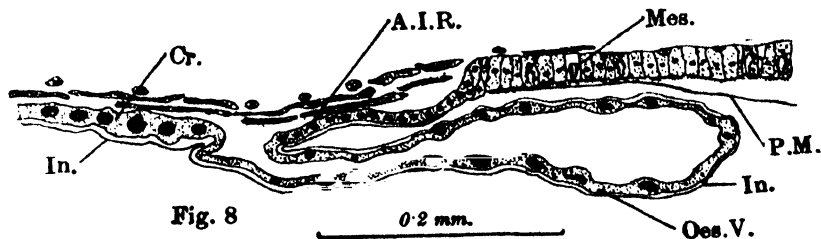
Fig. 8
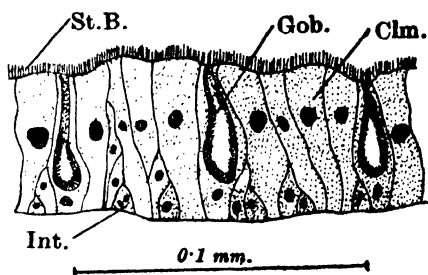
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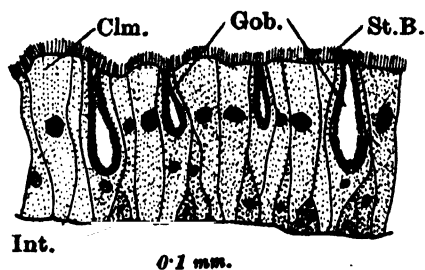
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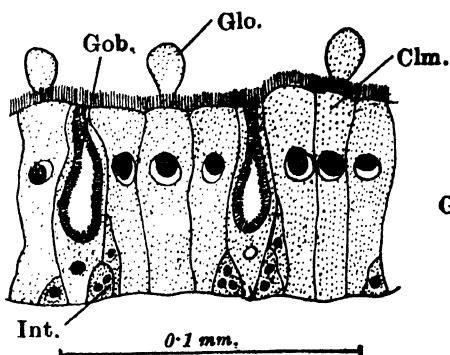
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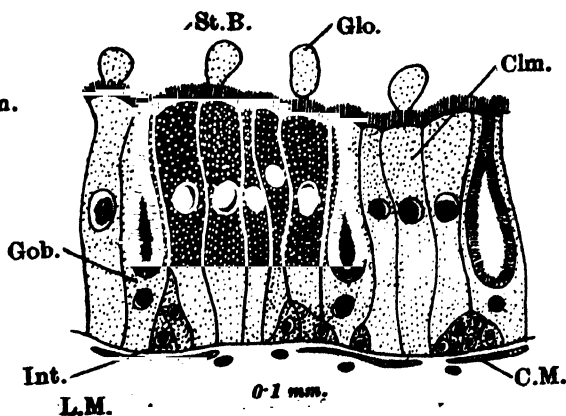
Fig. 12

Fig. 8, L.S. through the oesophageal valve; Fig. 9, T.S. midgut of early third instar larva; Fig. 10, T.S. midgut of third instar larva of a little later stage; Fig. 11, T.S. midgut of the late third instar larva; Fig. 12, T.S. midgut of fourth instar larva.

A.I.R., Anterior interstitial ring; *Clm.*, Columnar cell; *C.M.*, Circular muscles; *Cr.* Crop; *Glo.*, Globular extrusions; *Gob.*, Goblet cell; *In.*, Intima; *Int.*, Interstitial cell; *L.M.*, Longitudinal muscles; *Mes.*, Mesenteron; *Oes.V.*, Oesophageal valve; *P.M.* Peritrophic membrane; *St.B.*, Striated border.

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the columnar cells with their cytoplasm strongly stainable. Those which are only partially developed are narrow and possess a comparatively large nuclei. The goblet cells are all of uniform size.

In the middle aged third instar larvae (Fig. 10) the interstitial cells are fewer while the normal sized columnar cells are comparatively more numerous. These normal sized columnar cells are surrounded by many smaller ones which vary in size. The smaller cells obviously represent stages in the development of columnar cells and when fully developed will form the typical normal sized columnar cells. A few slightly taller cells also contain a small goblet and the total number of goblet cells is larger than the number found in the early third instar of the larvae. The presence of goblet cells of varying sizes also indicates that they have been recently formed. There appears to be no clear difference between the developing cells destined to form the columnar cells or the goblet cells. The fate of the newly formed cells is unpredictable until the appearance of a tiny goblet which distinguishes them from the columnar cells.

In the late third instar larvae (Fig. 11) i.e. just before the next ecdysis the epithelium possesses a large number of normal sized columnar and goblet cells. The number of interstitial cells is also larger. Although mitotic figures could not be observed, it is evident that the number is increased by the mitotic multiplication of the interstitial cells. Another noteworthy feature is the presence of a large number of globular protrusions or vesicles arising from the lumen ends of some of the older and normal sized columnar cells. These vesicles are attached to larger cells, particularly those which are far removed from the nests of interstitial cells and have been regarded by some early workers to be secretory in nature. But in view of the fact that they invariably appear in the midgut of the late instar of the larvae and arise only from older columnar cells they seem to be cell disintegration products. Had they been secretory in function they would have been more abundant in the gut of the early third instar of the larva when it feeds more actively than before the ecdysis.

(3) *The Peritrophic Membrane*

It is in the form of a thin transparent membrane (Fig. 8) surrounding the food contents in the lumen of the midgut. The whole of the membrane can be taken out along with the food contents which it envelops bringing with it a torn portion of the midgut cells of the anterior region. Close examination of the transverse and the longitudinal sections of the midgut reveals the total absence of any reinforcement of the membrane from the general surface of the midgut epithelium. Moreover, except for the anterior region where it is closely attached to the cells producing it, there is a definite space between the epithelium and the peritrophic membrane. This space is filled with scattered granular material. Hence it appears to be formed only by the cells of the anterior end of the midgut. The distance between the wall of the oesophageal valve and the midgut epithelium is many times greater than the thickness of this membrane. Hence there is no possibility of the valve working as a press to mould the peritrophic membrane as suggested by Wigglesworth (1930).

3. THE HINDGUT

The hindgut extends from the posterior end of the midgut to the anus (Fig 1). The layers of tissues in the hindgut are, (1) an enveloping membrane of connective tissue, (2) layers of muscle fibres, (3) a single layer of epithelium resting on basement membrane, and (4) a chitinous intima lining the epithelium internally. Six distinct regions can be recognized in the hindgut, viz. posterior interstitial ring, ileum, anterior sphincter region, colon, posterior sphincter region and rectum.

(1) *Posterior Interstitial Ring*

This region (Fig. 13) corresponds to the anterior interstitial ring of the foregut and is in the form of a double fold projecting into the lumen of the gut with both the muscular layers present. The cells of the ring are well defined but lack definite boundaries. The nuclei, which form a single row, are quite prominent and numerous. The chitinous intima lining the cells shows that they belong to the proctodeal regions. Henson (1931) in *Vanessa* found numerous transverse rows of fine bristles on the posterior layer of the intima. No such bristles could be detected in the insect under study.

(2) *Ileum*

Lying between the posterior interstitial ring and the anterior sphincter region is the funnel-like ileum. Its epithelium (Fig. 14) consists of a layer of large flat cells. The walls separating the cells are quite indistinct but the nuclei are easily visible. The cells in the anterior part of the ileum are larger and possess more distinct boundaries. The intima lining the epithelial cells of this region is quite smooth and uniform. The musculature consits of an inner layer of circular fibres which are covered over by an outer layer of longitudinal fibres.
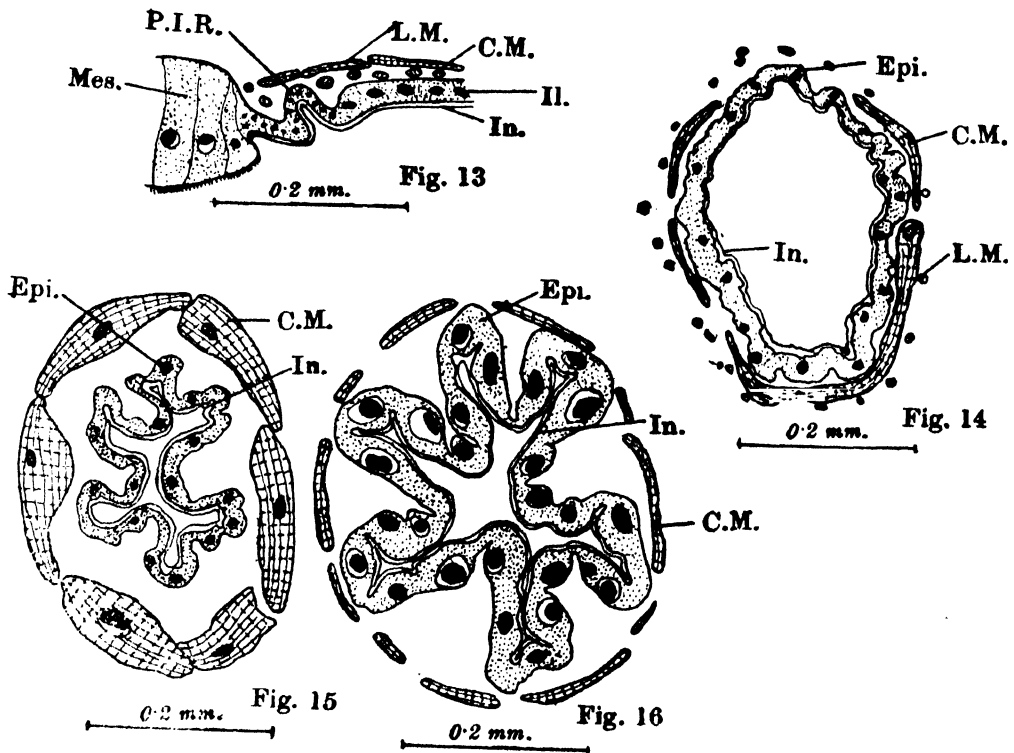
Fig. 13, L.S. through the junction of midgut and hindgut; Fig. 14, T.S. ileum; Fig. 15, T.S. anterior sphincter region; Fig. 16, T.S. colon.

C.M., Circular muscles; *Epi.*, Epithelium; *Il.*, Ileum; *In.*, Intima; *L.M.*, Longitudinal muscles; *Mes.*, Mesenteron; *P.I.R.*, Posterior interstitial ring.

(3) *The Anterior Sphincter Region*

Between the ileum and the colon is the tube-like anterior sphincter region. This region (Fig. 15) becomes prominent because of having very well developed musculature. The circular muscles are very strongly developed. The epithelial cells possess large prominent nuclei but are not provided with distinct cell boundaries. The wall of the epithelium is thrown into deep longitudinal folds.
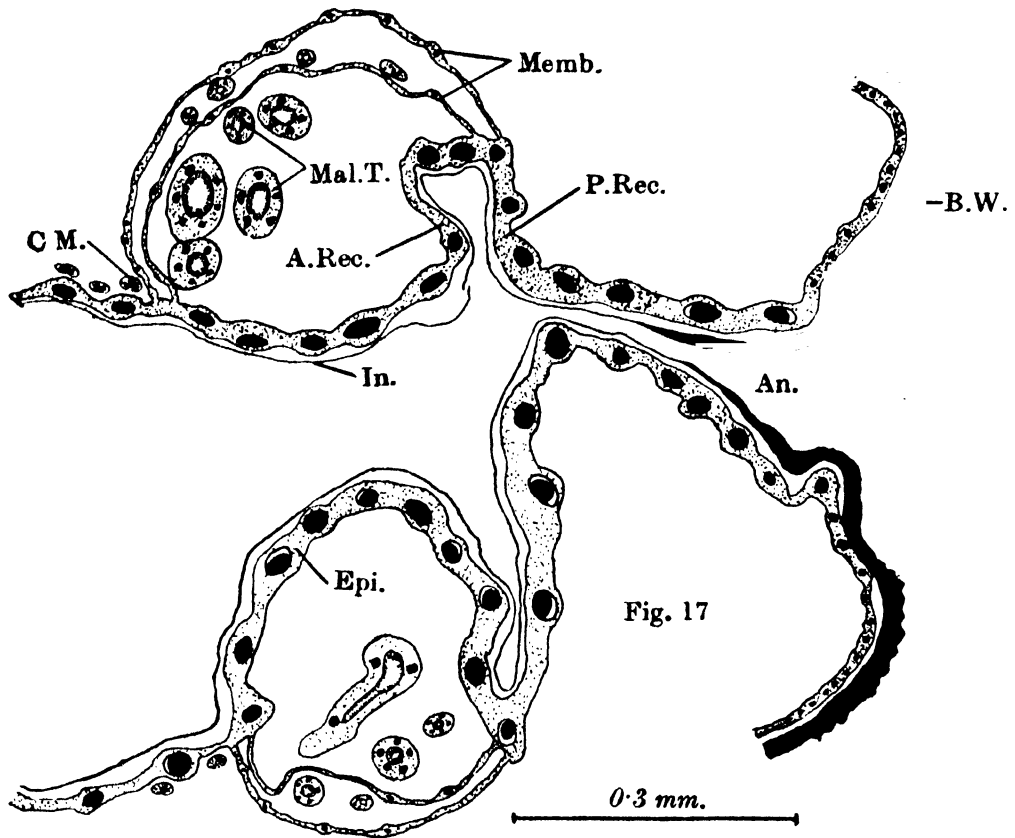
L.V.S. through rectum and fiscal chamber.

An., Anus; *A.Rec.*, Anterior rectum; *B.W.*, Body wall; *C.M.*, Circular muscles; *Epi.*, Epithelium; *In.*, Intima; *Mal.T.*, Malpighian tubule; *Memb.*, Membranes forming the fiscal chamber; *P.Rec.*, Posterior rectum.

(4) *The Colon*

It is a distinct globular structure lying between the anterior and the posterior sphincter regions (Fig. 1). The epithelium (Fig. 16) is well defined with large flat cells possessing prominent and deeply staining nuclei. The cells are not provided with distinct boundary lines. The wall of the colon is thrown into several longitudinal folds. The intima is smooth and thin and is not provided with bristles. The muscle system is much reduced and only the circular muscles are present.

(5) *The Posterior Sphincter Region*

Lying in between the colon and the rectum is the posterior sphincter region which is provided with strongly developed circular muscles. The epithelial wall is thrown into deep longitudinal folds. The cells have large and deeply staining nuclei and indistinct boundary walls, thus presenting a syncytial appearance. The intima is smooth and well developed. The posterior sphincter region is feebly developed in comparison with the anterior sphincter region.

(6) *The Rectum*

The rectum (Fig. 17) is differenciated into two parts—the anterior rectum and the posterior rectum.

The anterior rectum consists of the first half of the globular chamber. Its epithelium consists of very large flattened cells which possess large and deeply staining nuclei. The intima is smooth and is much similar to that of the colon. Surrounding the entire region of the anterior rectum are two thin membranes of squamous epithelium. The malpighian vessels lie in the space between the epithelium and the membranes.

The posterior rectum joins the anterior rectum obliquely and continues to the anus of the larva. The whole of the posterior rectum appears funnel shaped in the longitudinal sections and triangular in the transverse sections. The epithelial cells comprising the posterior rectum are smaller. The intima is smooth and possesses no bristles. The anterior margin of the posterior rectum is marked by the origin of the two membranes (Fig. 17) of the fiscal chambers which envelop the anterior rectum.

DISCUSSION

(1) *Oesophageal Valve*

Wigglesworth (1930) in *Cheimabacche fegella*, *Ephestia kuhniella* and *Sitotroga cerealella*, Henson (1931) in *Vanessa* and several others in various Lepidoptera have described the oesophageal valve to be hanging down in the midgut as a delicate curtain and not provided with blood sinus. The oesophageal valve in *L. orbonalis* is provided with a blood sinus which has so far not been reported in any other Lepidoptera except in *Io irene* (Bordas, 1911). In the insect under study the oesophageal epithelium is continued over the folds as also observed by Henson (1931) in *Vanessa*. The cells of the oesophageal valve are similar to those of oesophagus and are lined with chitinous intima which is neither provided with spines nor is thickened to form a conspicuous ring and is thus unlike those described by Wigglesworth (1930) in Diptera and Hymenoptera.

Henson (1931) felt that in *Vanessa* this structure is inefficient as a valve because it is not provided with restraining cords and also because it is incomplete like a split cylinder, being absent in the midventral line. He regards it as a mere guide for leading the food in the midgut. Wigglesworth (1930) however regards that in those Lepidoptera in which it is not provided with a sinus, the fold of the oesophageal valve serves to press the peritrophic membrane against the midgut epithelial cells producing it because in normal state the gut of the larva remains distended, whereas in those larvae in which a blood sinus is present the condition is similar to that of tenthredinid larva and the sinus itself gets distended to work as an efficient press. He stressed the hypothesis that the function of the invagination is to permit the peritrophic membrane to arise anteriorly to the point of entry of the food into the gut. Moreover, he believed that the peritrophic membrane is pressed between the invagination and the midgut epithelium. This

may well be true, for example, in Diptera and in Dermaptera where there is a conspicuous annular ring on the posterior edge of the invagination which might act as a press. But it certainly does not appear to be the case in *L. orbonalis* in which no such ring is present and the distance between the oesophageal invagination and the midgut epithelium is many times the thickness of the peritrophic membrane. Moreover, the invaginated organ must undergo the normal writhing movements which would render it unsuitable to act as a press. It, therefore, appears reasonable to believe that the oesophageal valve in *L. orbonalis* is not likely to function as an efficient press mechanism. Moreover, like *Vanessa* it is incomplete and without cords ; hence it cannot work as a valve also. Certainly some other function must be ascribed to the oesophageal invagination. A function, and an important one, of the crop is to get dilated with air during moulting to enlarge the cuticle before hardening occurs. A study of the crop in moulting insects reveals that it is the main organ distended with air and that there must be an effective mechanism to prevent this air from passing into the midgut. The oesophageal invagination appears to serve this purpose.

(2) *Midgut Epithelium*

The presence of three different kinds of cells, viz., interestial, columnar and goblet brings up the question of their morphological relationship with one another.

There is no doubt that the interstitial cells are really embryonic rudiments and the large numbers in which they are produced at each larval ecdysis clearly suggests that their primary function is to increase the number of the goblet and the columnar cells. Their regenerative function has been established by Henson (1929) in *Vanessa*, Yung Tai (1929*a*) in *Galleria* and Woke (1941) in *Prodenia*. In *L. orbonalis* also it has been found that in the early third instar larva there are a large number of interstitial cells which soon differentiate into the columnar and the goblet cells. Although multiplication by mitosis could not be difinitely observed but their appearance in very large numbers is evidently by mitotic division of the originally existing interstitial cells.

Regarding the morphological relationship of the columnar and the goblet cells some early workers notably Shinoda (1927) and Buchmann (1928) concluded that the two forms are homologous. Shinoda regarded the different forms of cells as functional variations of the same type and postulated that the function of the columnar cells is both secretory and absorptive while the goblet cells are developed from the columnar ones when the latter have emitted secretion. Vignon (1901), however, felt that the goblet cells are not a phase in the development of the columnar cells. Henson (1929), Tung Tai (1929*b*) and Woke (1941) believed that both the types of cells are present at the time of hatching and that they are distinctly different kinds of functional epithelial cells. They regarded them to be dimorphous. Yung Tai found that they are differentiated even in the embryo and that they are generated separately from the interstitial cells. Observations in *L. orbonalis* indicate that both the types of cells are dimorphous.

The question arises about the respective functions of the two kinds of cells. Notable among the early workers was Van Gehuchten (1890) who regarded that the functions of secretion and absorption are carried out by two sets of cells. Yung Tai (1929), on the other hand, postulated that the goblet cells of the larval epithelium of *Galleria* are exclusively secretory while the columnar cells may be either secretory or absorptive in function, though the same individual cells do not function in both the capacities. Woke (1941) believed that in *Prodenia* the columnar cells are both secretory and absorptive in function while the goblet cells may be only secretory. He feels that the cavities of the goblet cells may satisfactorily function as reservoirs of secretions so that it may readily reach the lumen through a narrow opening at its lumen end. In most of the insects, however,

the goblet cells are absent and both the functions of secretion and absorption are performed by the epithelial cells which very much resemble the ordinary columnar cells of the lepidopterous larvae. Hence, it appears reasonable to believe that the columnar cells of the larval Lepidoptera are also capable for both absorption and secretion. Although nothing can be definitely said about the possible role of the goblet cells, their morphological structure indicates that they are more suited for the purpose of secretion only, particularly because their surface which may be the only place for carrying on absorption is tucked inwards and does not come in direct contact with the food present in the lumen.

At the distal ends of many full sized and old columnar cells, globular protrusions or vesicles are observed. Van Gehuchten (1890), Bordas (1911) and Shinoda (1927) regarded these structures as secretion vesicles. This view was disagreed to by Pavlovksy and Zarin (1922) who believed that they are nothing more than artifacts. Henson (1929) proved that these vesicles are cell disintegration products. Yung Tai (1929) made a similar suggestion. This view has subsequently been shared by Woke (1941) who considered the globular protrusions as evidences of approaching metamorphosis. Day and Powning (1949) on the basis of both histological and physiological studies on *Blatta* showed that the presence of these vesicles has no relationship with the concentration of enzymes in the midgut. It has been noted in *L. orbonalis* that the globular vesicles are not present throughout the larval period but make their appearance during the late third instar larvae. Normally the larvae feed actively soon after the ecdysis and stop feeding in the latter part of each instar i.e. just before the moulting. Hence if the globules are regarded to be secretory their formation should be continuous all along the larval life and their number should be particularly abundant when the insect is actively feeding i.e. in the early part of each instar. But such is not the case. Besides this, it has been observed that the globules arise invariably from the older cells. It also indicates that they represent cell disintegration products and are not in anyway related to the process of secretion.

(3) *Peritrophic Membrane*

The literature on the peritrophic membrane of insects is very extensive and controversial. Much confusion prevails because of a commonly held view that the cells of the midgut cannot secrete chitinous membrane. Bordas (1911) concluded that the peritrophic membrane is formed by the secretion of a deeply staining 'ring of small cells' at the base of the oesophageal valve. But he does not state whether these cells belong to the foregut or the midgut. Henson (1931) showed that this ring belongs to the foregut but he argued that this region of foregut is lined with chitinous intima and therefore cannot be expected to secrete two different types of lining for the foregut and the midgut at the same time. He concluded that the entire peritrophic membrane is secreted by midgut cells. Soon after, Grambell (1933) and Butt (1934) postulated that the cells of the anterior end of the midgut arise from stomodaeum and are, therefore, capable of producing chitinous peritrophic membrane. Most of the workers, viz., Van Gehuchten (1890), Vignon (1901), Pavlovksy and Zarin (1922), Snodgrass (1925), Wigglesworth (1930), Henson (1931) and several others have shown that the midgut cells as such, although derived from mesenteron, can secrete chitin and it is not necessary for the cells to have stomodaeal origin to be able to secrete peritrophic membrane. In *L. orbonalis* also it has been found that the peritrophic membrane is produced by the midgut epithelium. Now two modes of the formation of peritrophic membrane have been described. In some insects, it is a tough tube consisting of one or more layers and is produced mainly by the anterior end of the midgut. In others it consists of a large number of thin layers which arise by the successive delaminations from the entire surface of the midgut epithelium. Snodgrass (1925) showed that

in honey bee the multilayered membrane is formed of layers produced by both the methods. He described the existence of a number of connections between the membrane and the midgut epithelium which act as reinforcements. Henson (1931) indicated that although the peritrophic membrane in *Vanessa* is formed by the secretion of the cells of the anterior midgut it may also be reinforced by the secretion from the cells of the midgut along the whole of its length. Van Gehuchten (1890) observed that in *Ptychoptera* the cells of only the anterior end of the midgut secrete the membrane. Vignon (1901), on the other hand, regarded that in *Bombyx mori* the peritrophic membrane is formed by delamination from the entire surface of the midgut epithelium. It has been shown in *L. orbonalis* that the membrane is secreted by the midgut cells localized in its anterior region and is not reinforced from the rest of the midgut cells as in *Vanessa* (Henson, 1931) or honey bee (Snodgrass, 1925). In the insect under study the membrane is separated from the midgut epithelium by a clear space except at anterior end of the midgut where it is in intimate contact with the cells producing it. No other connection was observed between the membrane and the rest of general surface of the midgut. In fact, whenever the membrane is pulled out it brings with it some portions of the anterior region of the midgut epithelium which is clearly suggestive of the fact that the cells so torn give rise to the membrane.

REFERENCES

Bordas, L. (1910). Les glandes cephaliques de chenilles de Lepidopteres. *Ann. Sci. nat.* (Zool.), **8**, 125–193.

———— (1911). L'appareil digestif et les tubes de Malpighi des larves des Lepidopteres. *Ibid.*, **14**, 191–273.

Buchmann, W. W. (1928). Uber die Zellveranderungen im Mitteldarm Wahrend der Scleretion. *Zool. Anz.*, **79**, (1–2), 223–243.

Butt, F. H. (1934). The origin of the peritrophic membrane in *Sciara* and the honey bee. *Psyche, Camb., Mass.*, **41** (2), 51–56.

Day, M. F. and Powning, R. F. (1949). A study of the process of digestion in certain insects. *Aust. J. sci. Res.*, (b), **2**, 175–215.

Gramboll, F. L. (1933). The embryology of the black fly *Simulium picties* Haw. *Ann. ent. Soc. Amer.*, **27**, 641–71.

Henson, H. (1929). On the development of the midgut in the larval stage of *Vanessa urticae* (Lep.). *Quart. J. micr. Sci.*, **73**, 87–106.

———— (1931). The structure and the postembryonic development of *Vanessa urticae*—The larval alimentary canal. *Ibid.* **74**, 321–360.

Pavlovksy, E. N. and Zarin, E. J. (1922). On the structure of alimentary canal and its ferments in the bee (*A. mellifeca*). *Ibid.* **66**, 509–551.

Shinoda, O. (1927). Contributions to the knowledge of intestinal secretion of insects. II.A comparative histocytology of the midintestine in various orders of insects. *Z. Zell forsch.*, **5**, 278–292.

Snodgrass, R. E. (1925). Anatomy and Physiology of the Honey Bee. MacGraw-Hill Publ.

Van Gehuchten (1890). Recherches histologigues sur l'appareil digestif de la *Ptychoptera contaminata*. *Cellule*, **6**.

Wigglesworth, V. B. (1930). The formation of the peritrophic membrane in insects with special reference to the larvae of mosquitoes. *Quart. J. micr., Sci.*, **73**, 597–616.

Vignon (1901). Recherches sur les epitheliums. *Arch. Zool. exp. gen.*, **9**.

Woke, A (1941). Structure and the development of the alimentary canal of the Southern army worm larva. *Tech. Bull. U.S. Dep. Agric.*, **762**.

Yung-Tai, Tschang. (1929a). Researches sur l'histogenese et l'histophysiologie de l' epithelium de l'intestine moyen ches un lepidoptere (*Galleria mellonella*). *Biol. Bull.*, **12**, 1–144.

———— (1929b). Sur l'origine de la membrane peritrophique dans l'intestin moyen de chiniless de Lepidopteres. *Bull. Soc. zool. Fr.*, **54**, 255–263.

NOTES ON THREE SPECIES OF ZYGNEMACEAE FROM SOUTH INDIA

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ABSTRACT

Three interesting forms of the family Zygnemaceae viz. *Zygnema czurdae* Randh., *Zygogonium kumaoense* Randh., *Z. talguppense* Iyengar are reported here. A peculiarity in the lateral conjugation of *Zygnema czurdae* by the formation of conjugation tubes and the formation of azygospores are described.

The object of this paper is to report three interesting forms of the Zygnemaceae from South India. *Zygnema czurdae* Randh. was collected by Dr. M. S. Randhawa from Pennar river, near Anantapur, Andhra State on August 15, 1958. The other two forms, viz. *Zygogonium kumaoense* Randh. and *Z. talguppense* Iyeng. were collected by Dr. K. M. Aiyappa from Votecolli range, Western Ghats, Coorg, during August—November, 1958. The specimens collected during August—September were infertile, while fertile material of both these forms was obtained in November, 1958.

1. *Zygnema czurdae* Randhawa 1936. Proc. Indian Acad. Sci. B, **4**(3) : 239–241, figs. 1–7 ; Randhawa, Proc. Indian Acad. Sci., B, 1938, **8**(3) : 137–138, fig. 22.

Vegetative cells are 19–22.8 μ broad and $1\frac{1}{2}$ to 4 times as long. Each cell has a pair of more or less rounded chloroplasts with a conspicuous pyrenoid in each. Both scalariform and lateral conjugation take place as in the type. However, the predominant mode of conjugation is lateral.

Scalariform conjugation : Some filaments show the normal type of scalariform conjugation with globose zygospores in the conjugation canal which becomes slightly distended (Text-fig.1).

Lateral conjugation : The neighbouring cells give out small tent-like projection (Text-fig.2). The protoplasts move towards the tip of the protuberances, the cross wall separating the two gametes ruptures and the protoplasts fuse (Text-fig. 3). The four chloroplasts are clearly visible in the zygospore (Text-fig 4, and 5). The zygospore is oval to reniform in shape, 26.6–38 μ in diameter and fills the whole of the conjugation canal area. The rupturing of the flattened basal part of the conjugating cells as seen in the type, was, however, not observed.

In the lateral conjugation, some peculiarity was observed in some cases, where the neighbouring cells give out tent-like protuberances which grow out into distinct conjugation tubes (Test-figs. 6 and 7). Very rarely signs of incipient physiological anisogamy were observed. In such cases, the protoplast of one cell appeared to be more active and the zygospore was formed towards the female cell (Text-fig.8). Azygospores were also seen in some filaments. The protoplast from each cell, instead of fusing, formed two azygospores (Text-fig. 9, a).

Habitat : Free-floating in Pennar river, near Anantapur, Andhra State, August 15, 1958.

2. *Zygogonium kumaoense* Randhawa 1940. J. India Bot. Soc., **19** (5 and 6) : 247–249, figs. 1–11.

Vegetative cells are 9.5–11.4 μ broad and 30.4–57 μ long. The cell wall is thick and refractive and almost every cell produces a rhizoid which is either one or two celled (Text-fig. 10). The chloroplast is ill-defined and appears like strands with two to four swellings.
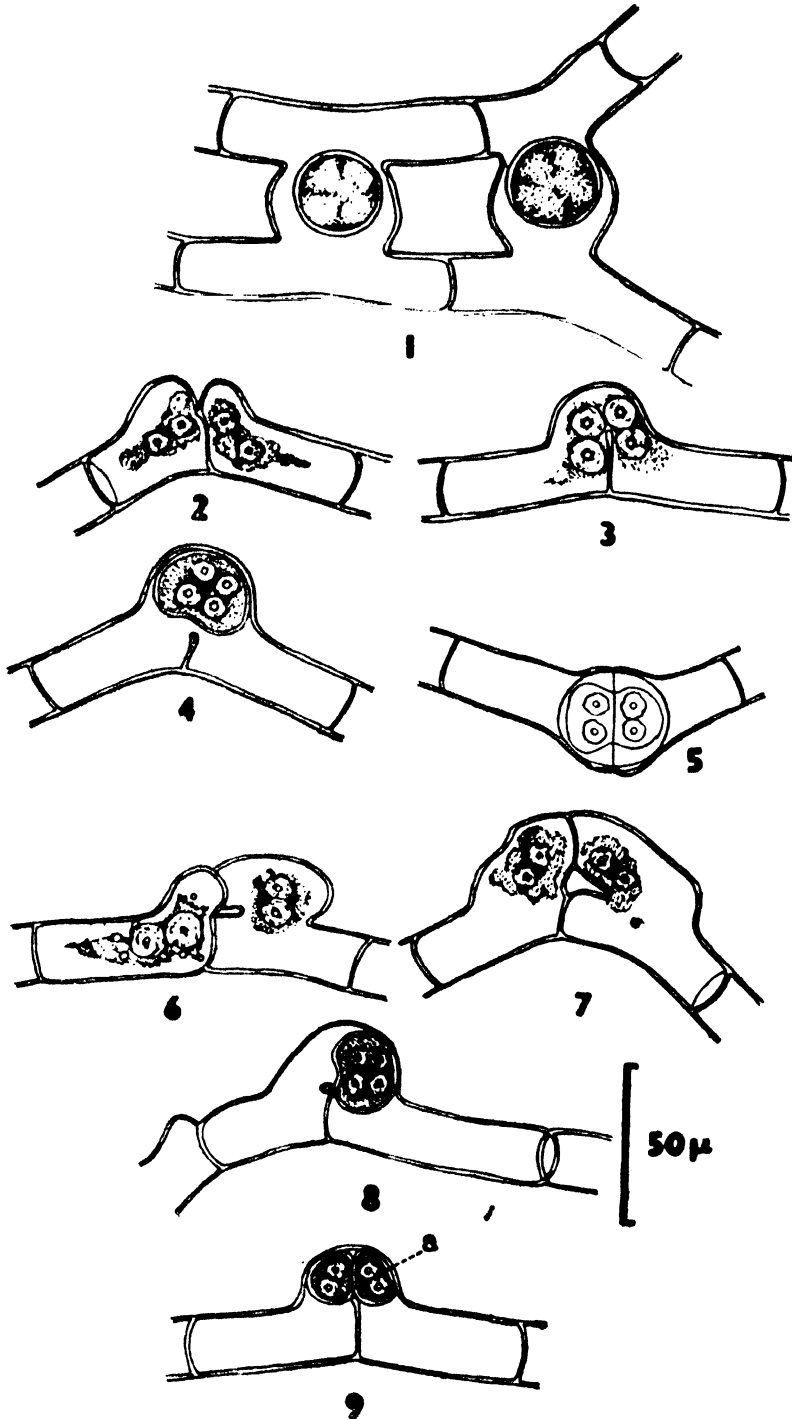
Zygnema czurdae Randh. Text-fig. 1. scalariform conjugation; Text-figs. 2-5, lateral conjugation (fig. 5, top view of the zygospore); Text-figs. 6-8, abnormal type of lateral conjugation by the formation of conjugation tubes; Text-fig. 8. Zygospore formed towards the female cell; Text-fig. 9. Azygospore (a).
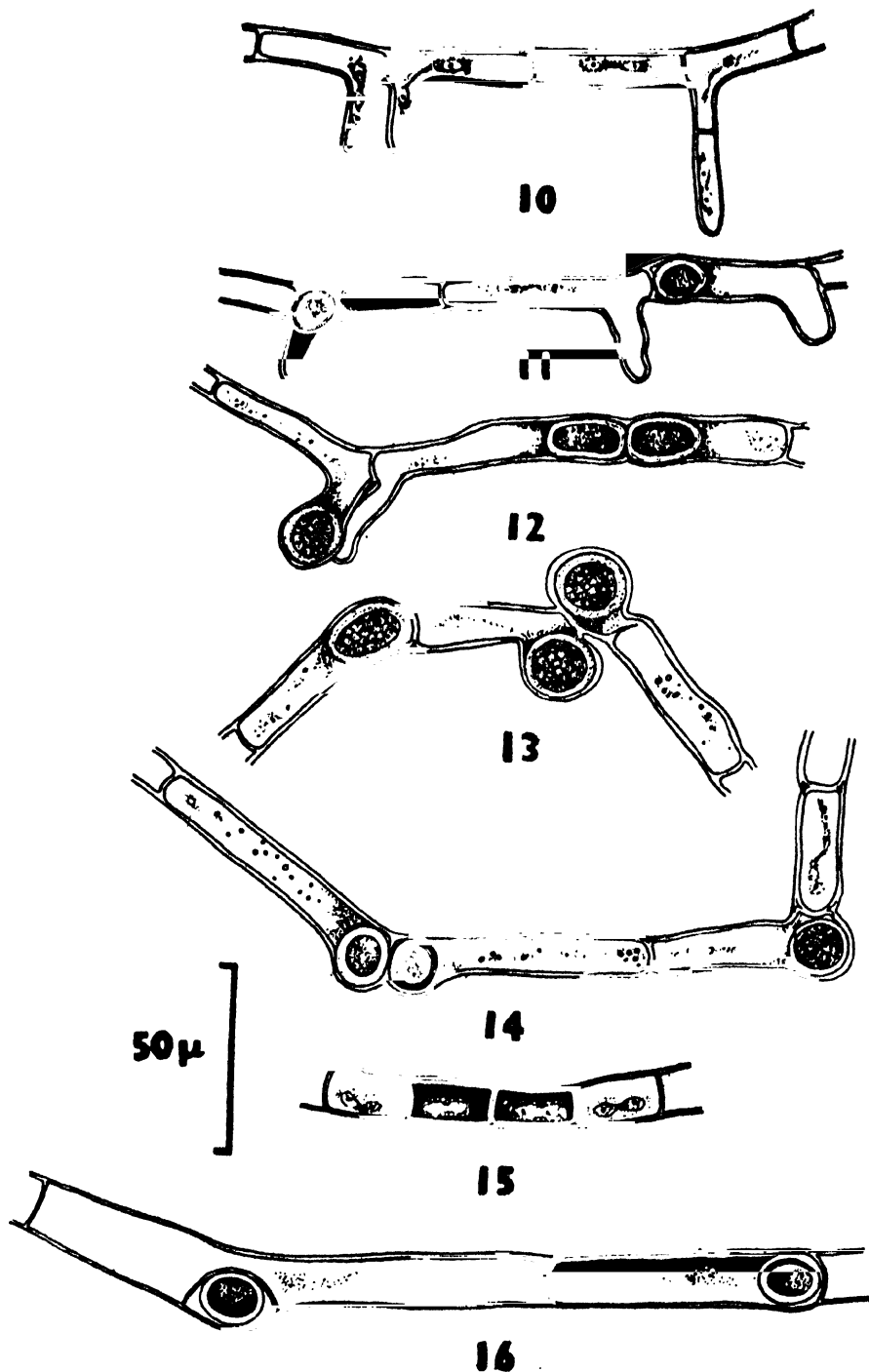
Zygogonium kumaoense Randh. Text-fig. 10, vegetative filament showing rhizoids Text-figs. 11–14, aplanospores in various positions; Text-figs. 15-16. *Zygogonium talguppense* Iyengar. Text-fig. 15. vegetative cells with chloroplasts; Text-fig. 16. portion of the filament with azygospores.

The reproduction is exclusively by aplanospores which are globose to ovoid, 9.3–10.3 μ broad, 11.4–15.2 μ long and are not cut off by any wall from the aplanosporangia. During the aplanospore formation, only a portion of the protoplast is utilised and protoplasmic remains are always left behind in the cell. The aplanospores are formed at the ends of the cells or sometimes at the tip of the rhizoids (Text-figs. 11–13). Occasionally the aplanospores of the adjacent cells are grouped in pairs on either side of the cross wall (Text-fig. 14). Very rarely, the aplanospores are also found outside the cells in the form of large swellings (Text-fig. 13). The spore wall is thick and faintly lamellated.

Habitat : On red loam soil on hill slopes along with *Zygogonium talguppense* Iyengar and *Z. ericetorum* Kütz., Votecolli range, Western Ghats, Coorg, August-November, 1958.

3.   *Zygogonium talguppense* Iyengar 1932. Rev. Algol., **6** : 263–267, fig. 1, a-d.

Vegetative cells 15.2–19.0 μ broad and 26.6–95 (–121.6) μ long. Each cell has a single axile chloroplast consisting of two rounded portions, each containing a pyrenoid (Text-fig. 15).

Reproduction is by azygospores. During the azygospore formation, the sporangium shows a slight swelling on one side into which most of the cell contents with the chloroplast and the nucleus pass, leaving behind a small portion of the protoplast. A curved wall is then formed cutting off the bulged portion as a lens-shaped cell (Text-fig. 16). The azygospores are ellipsoid to globose, 11.4–13.3 μ broad and 13.3–15.2 μ long. In some cases, the azygospores of the adjacent sporangia are grouped in pairs on either side of the cross wall.

Habitat : On red loam soil on hill slopes along with *Zygogonium ericetorum* Kütz. and *Z. kumaoense* Randhawa, Votecolli range, Western Ghats, Coorg, August-November, 1958.

REFERENCES

Iyengar, M. O. P. (1932).   Studies on Indian Zygnemales. *Rev. Algol.*, **6**, 263–267.
Randhawa, M. S. (1936).   Three new species of *Zygnema* from Northern India. *Proc. Indian Acad. Sci.*, *B* **4**(3), 239–241.
———— (1938).   Observations on some Indian Zygnemales from Northern India—Parts I & II. *Ibid.*, *B* **8**, 137–138.
———— (1940).   *Zygogonium kumaoense*, a new species of *Zygogonium* from Kumaon. *J. Indian bot. Soc.*, **19**(5 & 6), 247–249.

ADHESIVE APPARATUS OF A HILL-STREAM CYPRINID FISH
GARRA MULLYA (SYKES)

by Subhash Chandra Saxena, *Department of Zoology, Delhi University*

ABSTRACT

The paper deals with the functional morphology and histology of the integumentary modifications of *Garra mullya* (Sykes).

The adhesive apparatus consists of the modified lips, mentum and paired fins, which enable the fish to adhere to substratum in torrential streams. The histology of these parts along with that of the snout and thorax has been dealt with in detail.

Adhesion is performed suctorially by the callous portion of the disc. The lips, tuberculated border of the disc and the paired fins act as frictional device to prevent skidding, while the snout and thorax play only a secondary rôle in adhesion. The functional rôle of these organs has been attributed on the basis of their morphology and histology.

Due to the absence of any muscle in the tuberculated border, the process of attachment to the substratum and detachment from it, appears to be under some reflex control.

INTRODUCTION

The present paper deals with the adhesive modifications of the integument of a hill-stream cyprinid fish *Garra mullya* (Sykes) which belongs to the genus *Garra*, sub-family Cyprininae and family Cyprinidae. Genus *Garra* enjoys a wide distribution in Asia and Africa. However, the species *Garra mullya* (Sykes) is limited to the hills of peninsular India, Satpura Vindhya mountains and the Chota Nagpur Plateau.

One of the earliest papers on the torrential fishes of India was "Evolution of the adhesive apparatus in the hill-stream fishes" by Annandale (1919). Hora (1921 and 1922) described the systematics of the genus *Garra* and gave a comparative account of the structural and histological modifications in a few Cyprinoid and Siluroid hill-stream fishes ; *Garra annandalei* and *Glyptothorax* species were dealt with in detail. Hora (1952) also reported on the food and feeding habits of *Garra mullya* (Sykes). Rauther (1928) published a paper on the histology of mouth parts of two species of *Discognathus* (= *Garra*) emphasising their hill-stream adaptations. Wu and Liu (1940) described the "Adhesive Apparatus" of a Chinese Siluroid fish *Glyptothorax*. Bimla Bhatia (1950) gave an account of the epidermal adaptive modifications in *Glyptothorax telchitta*.

The material for investigation was sent in 1950 to the department of Zoology, University of Delhi, by the late Dr. S. L. Hora, Director, Zoological Survey of India. It was originally collected in 1948 from the shallow rivulets and pools of considerably fast currents of Aravalli Range of Rajasthan. Specimens ranging from 33 mm. to 90 mm. in standard lengths were fixed in formalin and preserved in 70 per cent alcohol. Sections were cut at $5\text{-}7\mu$ and were stained with haematoxylin-eosin or with Mallory's triple stain for connective tissue. Frozen sections were taken for histochemical detection of fat and silver impregnation for collagen fibres. The histological studies are mainly based on the adult specimens.

INTEGUMENTARY MODIFICATIONS

Gross Anatomy

The Cyrpinoid fishes found in the hill-streams of India are represented by genera belonging to the families Cyprinidae, Cobitidae, and Homalopteridae. These

fishes show a remarkable uniformity in their body contour. Dorsally the body is slightly arched, while ventrally the surface from snout to anus is usually flat.

The snout of *Garra mullya* is dorso-ventrally compressed, anteriorly rounded, smooth and coated with mucus. The mouth is located ventrally at about one third distance from the anterior tip. With the shifting of the mouth, the snout has extended to a ventral position. Mouth, crescentic in shape, is guarded by the heavily tuberculated, protractile anterior and posterior lips (Fig. 1). The anterior lip is more protractile than the posterior and has swollen corners. The posterior lip lies in front of the disc from which it is separated by a shallow groove. There is a pair each of maxillary and mandibular barbels.
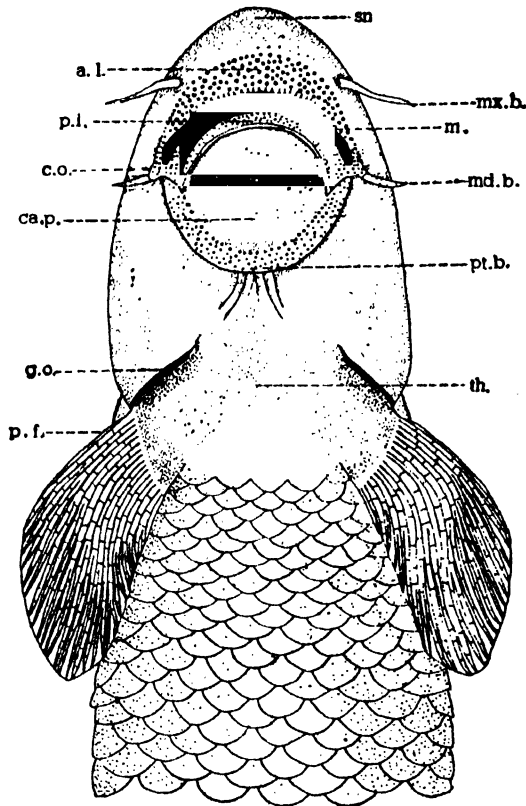
TEXT-FIG. 1.

Garra mullya (Sykes). Ventral view of head and trunk.
a. l., anterior lip; *ca. p.*, callous portion of the disc; *co.*, swollen corner of the anterior lip; *g. o.*, gill opening; *md. b.*, mandibular barbel; *m.*, mouth; *mx. b.*, maxillary barbel; *p. f.*, pectoral fin; *p. l.*, posterior lip; *pt. b.*, postero-lateral tuberculated border of the disc; *sn.*, snout; *th.*, thorax.

The disc, present in the mental region, consists of a central circular, smooth callous portion with an involuted postero-lateral tuberculated border. The involuted surface of the border is devoid of tubercles. The tubercles of the lips and those on the border of the disc bear microscopic spines. The relative position of the mouth-parts with the disc is shown in Fig. 2.

Garra mullya has very well developed cycloid scales over its body, but the thorax on the ventral surface is scaleless and smooth. The pectoral and pelvic fins are

more ventrally attached to the body. The fin rays are segmented and branched distally. In gross anatomy they do not show any modifications but histology of the integument reveals an interesting feature which is described later.
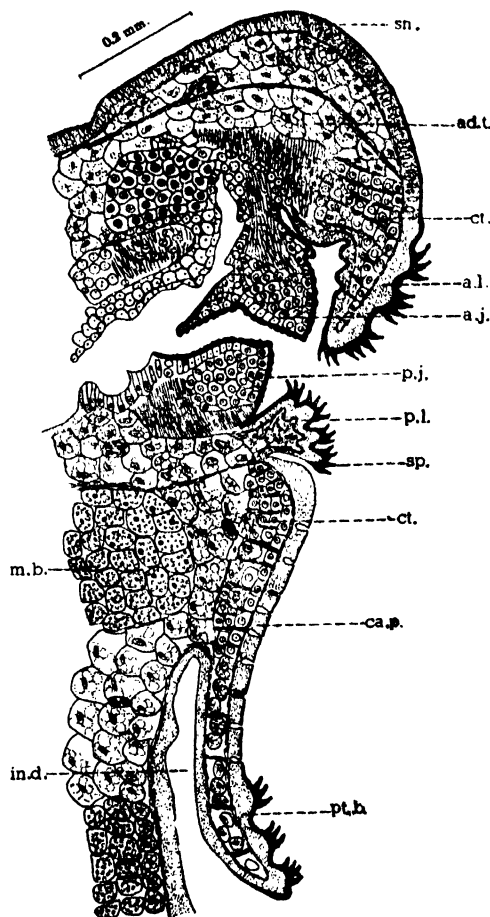
TEXT-FIG. 2.

Garra mullya (Sykes). Longitudinal section of the head showing relative position of mouth parts and disc from specimen of 50 mm. standard length.

a. l., anterior lip; *a. j.*, anterior jaw; *ad. t.*, adipose tissue; *ca. p.*, callous portion of the disc; *ct.*, modified connective tissue; *in d.*, involution of the disc; *m. b.*, muscle bundle; *p. j.*, posterior jaw; *p. l.*, posterior lip; *pt. b.*, postero-lateral tuberculated border of the disc; *sn.*, snout; *sp.*, spine.

HISTOLOGY

The Snout

The epidermis of snout (Fig. 3*a*), covered by a thin stratum corneum, consists mainly of oval, thick-walled epithelial cells, among which are embedded myriads of mucus secreting cells. The cells of stratum germinativum are distinct from the rest and are columnar with dense cytoplasm and oval nuclei. In the basal epidermal layers are large clavate cells with distinct nuclei and homogenous cytoplasm which stains with difficulty. The dermis is composed of many layers of

what seems to be adipose tissue. A narrow belt of collagen fibres, stratum compactum, cements the dermis with the epidermis. To determine the true nature of the tissues of dermis, frozen sections were cut and examined. In unstained sections a yellow substance that fills up spaces gave a positive reaction with Sudan III and Sudan Black B, thereby confirming the presence of fat.
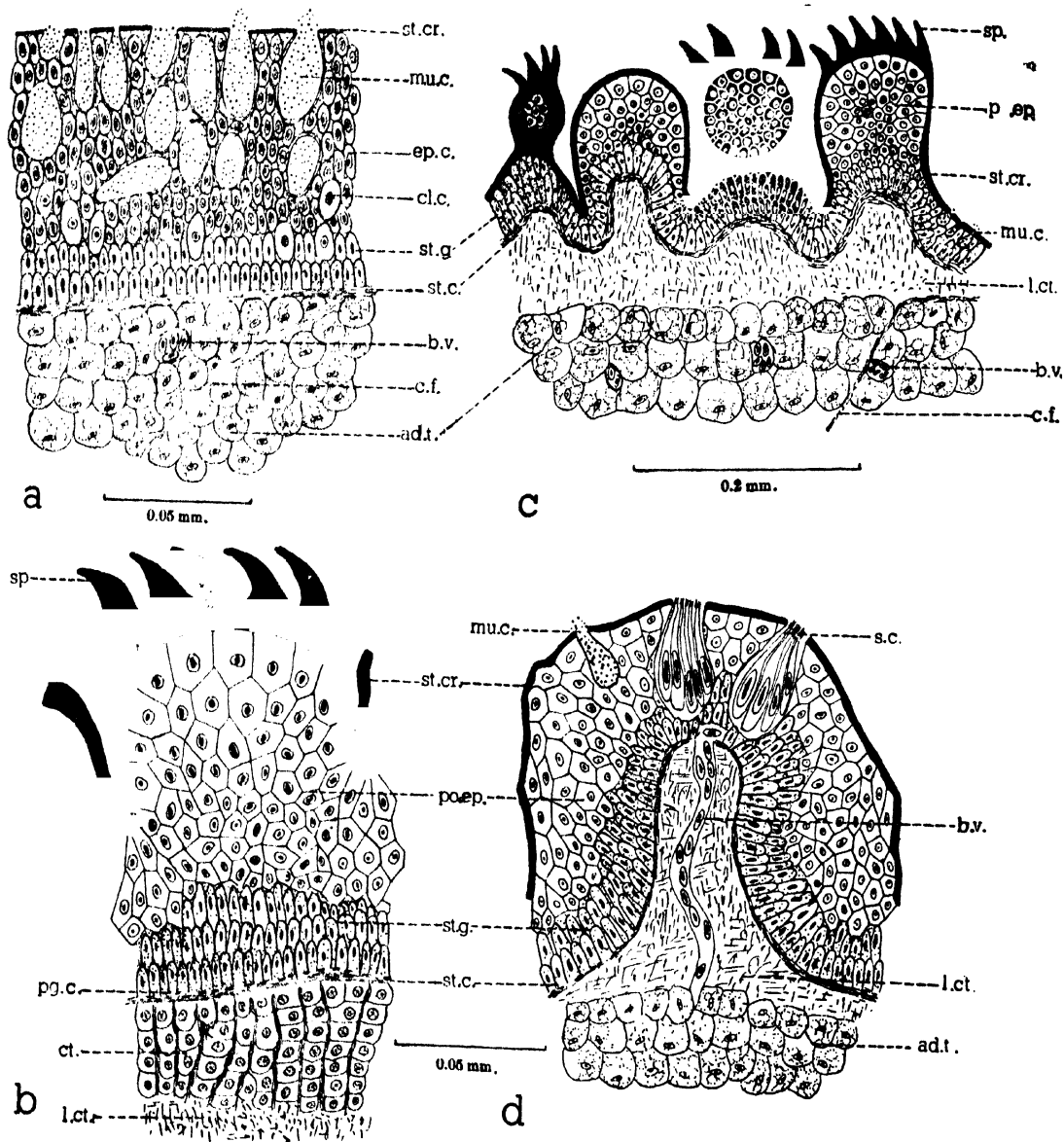
TEXT-FIG. 3.

Garra mullya (Sykes). *a.* Longitudinal section through the integument of snout; *b.*, Transverse section through the tubercle of anterior lip; *c.* Transverse section through the tubercles of posterior lip; *d.* Transverse section through the tubercle of posterior lip from specimen of 43 mm. standard length.; *a. b.* and *c.*, sections from specimens of 90 mm. standard length. *ad. t.*, adipose tissue; *b. v.*, blood vessel; *c. f.*, collagen fibres; *ct.*, modified connective tissue; *cl. c.*, clavate cell; *ep. c.*, oval epithelial cells; *l. ct.*, loose connective tissue; *mu. c.*, mucous cell; *pg., c.*, pigment cell; *po. ep.*, polygonal epithelial cells; *s. c.*, sensory cell; *sp.*, spine; *st. c.*, stratum compactum; *st. cr.*, stratum corneum; *st. g.*, stratum germinativum.

The Anterior Lip

The anterior lip is densely beset with tubercles. The stratum corneum extends over the tubercles to form spines, which are not present in grooves between the adjacent tubercles. Each tubercle (Fig. 3b), is packed up with polygonal epithelial cells, having clear cytoplasm and centrally placed big nuclei. The cells of stratum germinativum are distinct from the rest of the epithelial cells by their columnar appearance, dense cytoplasm and big oval nuclei. Sensory and mucous cells in the labial epidermis of young fish (33 mm. standard length), are absent in the adult (90 mm. standard length). The epidermis rests on fibrous stratum compactum. Pigment cells are present in between the stratum compactum and stratum germinativum. Below the stratum compactum is a distinct zone of modified and compactly arranged connective tissue cells, which are knit together by collagen fibres. This arrangement of connective tissue is quite peculiar. Under this zone lies the loose connective tissue.

The Posterior Lip

The tubercles of the posterior lip, unlike those of the anterior lip, are formed both by epidermis and dermis (Fig. 3c). The epidermis conforms in structure to that of the anterior lip. However, mucous cells are present on the corners of the lip, but in young fishes (43 mm. and 50 mm. standard length), the sensory and mucous cells are prominent on the tubercles (Fig. 3d). These tubercles are covered by thin stratum corneum which usually does not form spines. Each cluster of sensory cells is supplied with a blood vessel at its base. Dermis consists of loose connective tissue supported below by adipose tissue.

The Disc

The epidermis of the central callous portion (Fig. 4) extends over the marginal tuberculated border. Stratum corneum on the callous portion is thin but it becomes thicker over the tubercles, eventually giving rise to spines. A large number of mucous cells and a few sensory and clavate cells are present on the callous portion, while the tuberculated border is devoid of such cells.

The dermis of the callous portion consists of modified compactly arranged connective tissue cells knit together by collagen fibres. Below the modified connective tissue lie a few adipose tissue cells. In the tuberculated border, the dermis consists of strips of collagen fibres that form a pad-like swelling below each tubercle. The space between the adjacent strips of collagen fibres is filled with adipose and loose connective tissues. In the centre of callous portion is inserted a muscle which is attached to urohyal of the branchial skeleton.

Histological study in serial sections of the disc in young fishes (33 mm., 43 mm. and 50 mm. standard lengths), reveals that the anteriormost callous portion of the disc is devoid of mucous cells but has sensory cells. Towards the centre both the sensory and the mucous cells are present, but posteriorly they diminish in number. The clavate cells are uniformly present in the epidermis of callous portion.

Dermis of the disc shows gradual modifications with increase in the standard length. In a fish of 43 mm. standard length, the whole dermis of the disc consists of adipose tissue, with strips of collagen fibres in the tuberculated border (Fig. 5a). In fishes of 50 mm. and 72 mm. standard length, the adipose tissue is partly replaced by a modified connective tissue in the callous portion and by the collagen fibres in tuberculated border. The collagen fibres in a fish of 90 mm. standard length become much prominent and extend for a short distance in the dermis of the callous portion.

6

The Thorax

Histological study of the skin of thorax shows the presence of low, narrow, longitudinal ridges in the central portion, the lateral areas being devoid of such modifications.

The epidermis of thorax is composed of loose epithelial cells. The sensory and mucous cells lie embedded in the epidermis. The dermis consists of adipose tissue and collagen fibres.
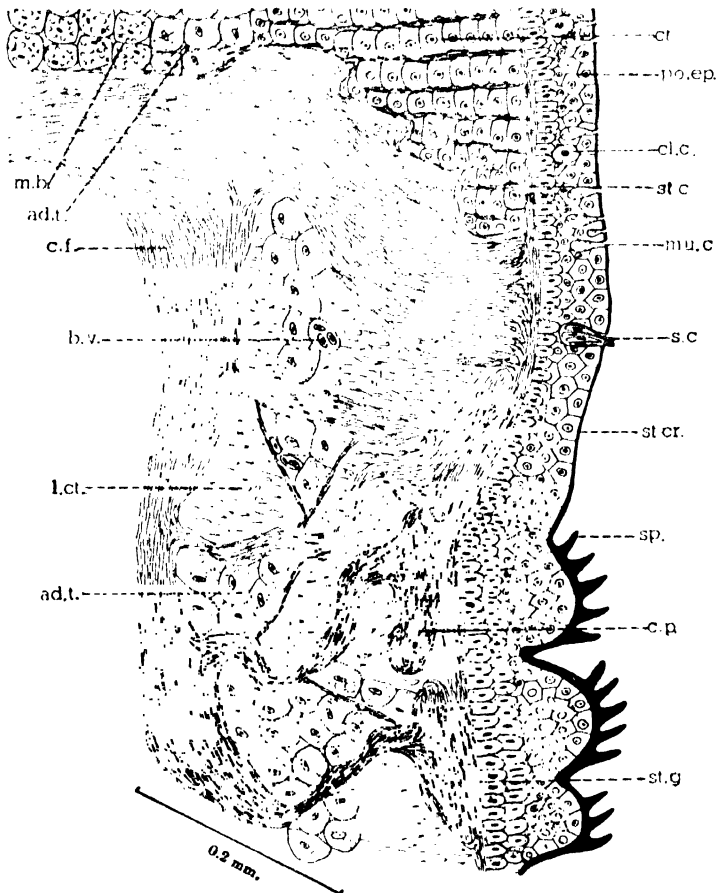
TEXT-FIG. 4.

Garra mullya (Sykes). Longitudinal section through the callous portion and tuberculated border of the disc from specimen of 90 mm. standard length.

ad. t., adipose tissue; *b. v.,* blood vessel; *c. f.,* collagen fibres; *c. p.,* collagen pad; *cl. c.,* clavate cell; *ct.,* modified connective tissue; *l. ct.,* loose connective tissue; *m. b.,* muscle bundle; *mu. c.,* mucous cell; *po. ep.,* polygonal epithelial cells; *s. c.,* sensory cell; *sp.,* spine; *st. c.,* stratum compactum; *st. cr.,* stratum corneum; *st. g.,* stratum germinativum.

The Paired Fins

Epidermis and dermis of the body integument extend over the pectoral and the pelvic fins. The modification of integument of the paired fins is mainly associated with the development of spines on the ventral surface of the first two fin rays (Fig. 5b). There are no spines over the other fin rays.

The Development of Tubercle and its Spines

During the course of investigation on *Garra mullya* of various standard lengths, it was possible to find the different developmental stages of tubercles and spine. Histological study shows that the development of tubercle on the anterior lip and the border of the disc is almost alike but differs from that of the posterior lip. On the anterior lip and the border of the disc, the rapid divisions of epidermal cells of stratum germinativum form papilla like structure, which protrudues out till a definite tubercle is formed ; but on the posterior lip (Fig. 3c, d) it is initiated by the outpushing of the dermis.
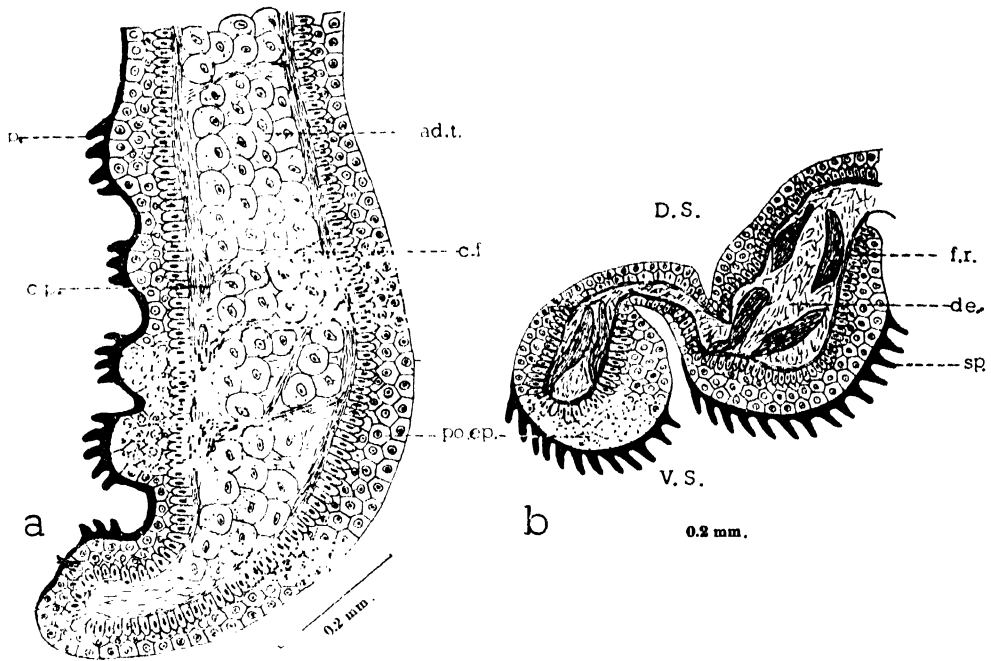
TEXT-FIG. 5.

Garra mullya (Sykes). *a*. Longitudinal section through the border of the disc showing formation of collagen pads from specimen of 43 mm. standard length; *b*. Transverse section of the pectoral fin showing the first two fin-rays from specimen of 72 mm. standard; length.

ad. t., adipose tissue; *c. f.*, collagen fibres; *c. p.*, collagen pad; *de.*, dermis; *D. S.*, dorsal surface; *f. r.*, fin-ray; *in. s.*, involuted side of the border; *po. ep.*, polygonal epithelia cells; *sp.*, spine; *V. S.*, ventral surface.

The development of spines is similar on all the organs of adhesion i.e. the lips, border of the disc and the fin rays of the paired fins. The spines are formed by the utermost layer of the epithelial cells, lying immediately below the stratum corneumo.

The cytoplasm of each peripheral cell (Fig. 6*a*, *b*) becomes differentiated into, outer thick and inner thin areas with the result that the cell wall protrudes out to form a conical projection. After the formation of conical projection, the nucleus of each peripheral cell divides and one of the daughter nuclei migrates into the projection (Fig. 6*c*). Soon, the chemical changes in the cytoplasm of each outer projection take place and transform it into a cornified spine (Schafer, 1949). The nucleus
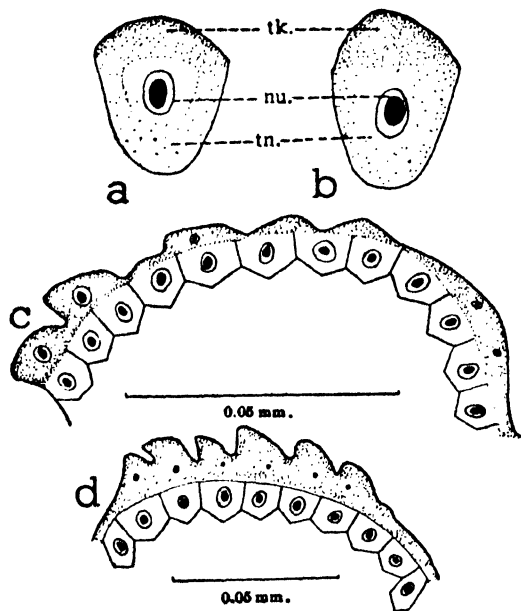
TEXT-FIG. 6.

Garra mullya (Sykes). Developmental stages of spine. *a*. and *b*. Two peripheral cells showing the outer thick protruding and inner thin area; *c*. Peripheral cells of a tubercle of posterior lip showing a few developing spines; *d*. Peripheral cells of a tubercle of anterior lip showing partially cornified spines From specimen of 43 mm. standard length.
tk., thick; *tn.*, thin; *nu.*, nucleus.

disappears in the process of cornification but it is seen distinctly in the partially cornified spines (Fig. 6*d*). The spiny layer breaks off at intervals due to wear and tear and is soon replaced by the underlying layer of the epithelial cells.

DISCUSSION

Our knowledge of the functional morphology of the lips and disc of the genus *Garra*, is based on the investigations of Hora (1922) and Rauther (1928). Rauther in his paper made no mention of Hora's previous detailed work on this genus. Their interpretations of the adhesive mechanism of the disc are mainly based on its external morphology. By the present study of the morphology and histology of the snout, the lips, the disc, the thorax and the paired fins of *G. mullya* it has been possible to determine the exact functional rôle of these organs in adhesion.

The snout of *G. mullya* conforms more or less in shape to that of less adapted hill-stream fishes such as *Crossocheilus* and *Schizothorax*. The abundance of mucous cells in the epidermis of snout of *G. mullya* is remarkable, which renders the fish with an oily appearance (Hora, 1952). Thus, the dorso-ventrally compressed form of the snout with mucous coating, offers minimum resistance to water current.

The study of the anterior and posterior lips reveals that both the lips having the same function, differ considerably in structure from each other. The histological differences exist primarily in the dermis of the lips. The dermis of the anterior lip consists of modified connective tissue (Vesikulöses Stützgewebe of Rauther, 1928), while in the posterior lip, it is composed of loose connective tissue and adipose tissue. The adipose tissue in the ordinary sections presents a reticulate appearance but is identified in frozen sections, by its reaction with Sudan III and Sudan Black B. Presumably, in the dermis of the posterior lip of *Discognathus blanfordi* Rauther could not detect its presence and hence named as "bindegewebe" (binding texture). Further, while dealing with the histology of the lips of *D. lamta* and *D. blanfordi*, he has not described the stratum compactum layer, which is a characteristic feature of the fish integument. The very layer in the lips of a Siluroid fish, *Glyptothorax telchitta*, has been described by Bimla Bhatia (1950), as a circular layer of muscles. In fact, this layer is composed of collagen fibres which stain blue in Mallory's connective tissue stain and brown in Silver impregnation, but in haematoxylin eosin stain, it takes a pink colour and gives a false appearance of muscles.

The protractile nature of the anterior lip shows that during adhesion, it has to bear more strain (maximum on its middle part, the mouth being crescentic), than the posterior lip, and the modified connective tissue appears to have secondarily developed as it is present only in the dermis of the protrusible part of the lip. The rigid and constant contact of the anterior lip with the substratum has resulted in the swelling of its corners, which have been interpreted as "connectives" by Hora and "rudiments of true lips" by Rauther. The presence of thick, spiny cornified layer over the tubercles shows the primary function of the lips to be adhesive, the tubercles and spines acting as frictional device. This fact is confirmed by the small number of sensory cells and their gradual disappearance from the lips of the adult fish. Rauther has assigned only a protective function to this cornified layer of the lips. He further states that the mouth may help in adhesion, by acting suctorially, the suction being maintained by the respiratory current. The suctorial force can only function in adhesion, provided there is a complete or partial vacuum. The respiratory current cannot bring about the suctorial force, however steady it may be, as water passes out through the gill openings.

The disc with its round shape, smooth shallow depression in the centre, is an admirable device for adhesion. It is the callous portion of the disc which forms an effective sucker when fish rests on the substratum. Contraction of the muscles under the callous portion forms a cavity with vacuum inside. The vacuum is maintained by the smooth margin of the callous portion (where the callous part merges into the tuberculated border), and not by the border as Hora suggested. The film of mucus formed along the margin further increases the adhesive power of the disc. The lips and border of the disc which act as frictional devices get fastened to the substratum and minimise chances of slipping. The tubercles and spines of the border do not allow it to keep a perfect contact with the substratum, but at the same time the row of collagen pads present below the tubercles render the border with tensile strength to adhere on the surface to serve as frictional device. As the cornified layer is of no adaptational value to the callous portion, it is very thin and can be detected only under very high magnification. But it becomes very thick and spiny over the border to increase friction. Dermis of the callous pad consists of the same modified tissue as that of the anterior lip. Thus, it is apparent that histological modifications of the callous portion and the border have a close correlation with their respective functions.

The lips are thrust against the substratum by the contraction of certain cranial muscles (Al-Hussaini, 1949 ; Girgis, 1952 ; Takahasi, 1925). Function of the callous portion is also under the control of the muscle. In the absence of muscles in the border, it can be surmised that it acts under some reflex control.

Owing to frequent adhesion and friction with the substratum, the thorax becomes flat and it is scaleless ventrally. The formation of incipient ridges in its central portion is in response to friction, and it plays only a secondary rôle in adhesive mechanism. The perching habit of the fish has brought about the ventral attachment of the paired fins to provide a large area for adhesion. Spines on the ventral surface of the first two fin rays further add to the mechanism of adhesion.

To summarise, adhesion is performed suctorially by the callous portion of the disc while the lips, postero-lateral border of the disc and the paired fins have only a frictional function. The snout and the thorax play a minor rôle only. When the fish detaches itself, it first releases the border from the substratum, and relaxes the muscle underlying the callous portion. Thus, the vacuum produced at the time of adhesion is disturbed and the fish swims away.

ACKNOWLEDGEMENT

REFERENCES

Al-Hussiani, A. H. (1949). On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits : Anatomy and histology. *Quart. J. micr. Sci.*, **90**,(2), 109.

Annandale, N. (1919). Evolution of the adhesive apparatus in hill-stream fishes. *Rec. Indian Mus.*, **14**, 117.

Bhatia, B. (1950). Adaptive modifications in a Hill-stream catfish *Glyptothorax telchitta* (Hamilton). *Proc. nat. Inst. Sci. India*, **16**, No. 4, 271.

Cowdry, E. V. (1948). Laboratory Technique in Biology and Medicine. The Williams & Wilkinsons Company, Baltimore.

Girgis, S. (1952). The bucco-pharyngeal feeding mechanism in the herbivorous bottom-feeding cyprinoid, *Labeo horie* (Cuvier). *J. Morch.*, **90**(2), 317.

Hora, S. L. (1921). Indian Cyprinoid fishes belonging to the genus *Garra*. *Rec. Indian Mus.*, **22**, 635.

———— (1922). Structural modifications in the fish of mountain torrents. *Ibid.*, **24**, 31.

———— (1930). Ecology, Bionomics and evolution of the Torrential fauna, with special reference to the Organs of Attachment. *Phil. Trans.*, B **219**, 171.

———— (1952). Species of fish referred to the Ramayana. *J. Asiat. Soc. (Letters)*, **18**(2), 63.

Mathur, B. B. L. (1953). Notes on Fishes from Rajasthan, India. *Rec. Indian Mus.*, **50**, Part 1, 105.

Rauther, M. (1928). Der Saugmund von *Discognathus*. *Zool. J.*, Abt. 3, **45**, 45.

Schafer, E. S. (1949). Schafer's Essentials of Histology. 15th Edition, Longman's, Green & Co., London.

Takahasi, N. (1925). On the homology of the cranial muscles of the Cypriniform fishes. *J. Morph.*, **40**, 1.

*Wu, H. W. and Liu, C. K. (1940). On the structure of the "Adhesive Apparatus" of *Glyptosternum*. *Sinensia*, **11**, 69.

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*Not referred in original.

PHOTOSYNTHESIS IN *HYDRILLA*

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ABSTRACT

The mechanism of utilization of organic acids in light in absence of CO_2 by *Hydrilla verticillata* has been studied using $C^{14}O_2$, $NaHC^{14}O_3$, acetate-1-C^{14}, acetate-2-C^{14} and malic acid-2, 3-C^{14}. Evidence is presented to show that malic acid is utilized in light as a substitute for CO_2 in photosynthesis in two different ways : (i) by decarboxylation to CO_2 which is then fixed along the conventional photosynthetic path with the evolution of O_2 which comes from water participating in the reaction, and (ii) by a conversion of malate to carbohydrate through phosphoenol pyruvate. The rate of decarboxylation as indicated by CO_2 evolution may be higher than that of CO_2 fixation and such acids as citric, succinic, fumaric, α-ketoglutaric, aspartic and glutamic may also be used in the same way.

The utilization of organic acids as a substrate for photosynthesis by a number of succulent plants has been a matter of controversy for several decades (Rabinowitch 1945). It has been suggested that the acids are reduced photochemically to carbohydrates directly or by a primary oxidation to CO_2 which is then fixed along the conventional photosynthetic pathway. Bose (1924) argued that it was "unreasonable to suppose that when the plant can assimilate or reduce so highly oxidised an acid as H_2CO_3 necessitating a large expenditure of energy it should not reduce the less oxidised organic acids". Working with *Hydrilla verticillata* an aquatic plant which accumulates acids in summer months, he was able to show that malic acid could completely substitute for CO_2, and photosynthesis—as evidenced by evolution of O_2—could continue in total absence of CO_2. The availability of isotopic tracers has made possible a re-investigation of the problem and a systematic investigation was undertaken, the results of which are presented below.

MATERIALS AND METHODS

Hydrilla verticillata was grown in the tanks of the Bose Institute. Before each experiment the twigs were washed thoroughly with sterile distilled water and cut into small pieces. Only green apical regions were used.

$BaC^{14}O_3$, $NaHC^{14}O_3$, Acetate-1-C^{14}, Acetate-2-C^{14} and Fumaric acid-2, 3-C^{14} were purchased from the Radiochemical Centre, Amersham, England. The $C^{14}O_2$ was generated by the action of lactic acid on $BaC^{14}O_3$ inside the experimental jar. Malic acid-2, 3-C^{14} was prepared from fumaric acid-2, 3-C^{14} by the method of Raha and Sen (1958).

At the end of each experiment the tissues were extracted in hot 80 per cent ethanol. The extract was filtered and the residue washed repeatedly with hot 80 per cent ethanol until the filtrate was free from any radioactivity. The combined filtrates were evaporated to dryness under a draft of air, dissolved in a small volume of distilled water and centrifuged. An aliquot of the clear supernatant was chromatographed two-dimensionally on Whatman no. 1 filter paper with water-saturated phenol and n-butanol-acetic acid-water (4 : 1 : 1) as the developing solvents (Sen and Leopold, 1956). Radioautographs of the chromatograms were prepared by exposure to Ilford or Kodak X-ray films for 3-8 weeks depending on the amount

of radioactivity present in the sample chromatographed. Radioactivity measurements were taken with an end window Nuclear Chicago halogen] counter connected with a Tracerlab Autoscaler. The compounds were identified from their migration characteristics in a variety of solvent systems and by co-chromatography, wherever possible. Starch was isolated according to the method of Calvin *et al.* (1949).

Experiments on the evolution of carbon dioxide were performed in closed Warburg vessels with N NaOH in the central well. The $C^{14}O_2$ trapped in the alkali was estimated as $BaC^{14}O_3$ and its specific activity determined. In experiments with non-radioactive organic acids of the Krebs cycle, the gas volumes were measured manometrically.

EXPERIMENTAL

The normal products of CO_2 fixation in Hydrilla.

The preliminary experiments were concerned largely with a study of the products of photosynthetic CO_2 fixation in this aquatic plant, with a view to finding out whether there is any deviation from the conventional path of carbon in photosynthesis demonstrated in a variety of plant tissues. *Hydrilla* twigs were incubated with 30μ C of $NaHC^{14}O_3$ (sp. activity 3.7 mg/mC) in phosphate buffer of pH 6.8 for 15 sec., 1 min., 5 min. and 15 min. At the end of each period the twigs were killed and extracted in boiling 80 per cent ethanol followed by paper chromatography and autoradiography. The distribution of C^{14} among the products of photosynthesis for a 15 sec. period is shown in Table 1. Bulk of the C^{14}-activity was located in the "nucleotide area" of the paper chromatograms and phosphoglyceric acid. No appreciable radioactivity could be detected in malic acid. Label in sucrose, malic acid and alanine appeared later and activity increased with increase in time. Similar results were also obtained with gaseous $C^{14}O_2$. The path of CO_2 fixation in *Hydrilla* thus appears to be normal.

TABLE I

The distribution of C^{14} in 15 sec. photosynthesis in Hydrilla verticillata

Compound	% total C^{14}-activity
"Nucleotide area"	55
Sugar phosphates	14
Phosphoglyceric acid	31

Utilization of dark CO_2 fixation products.

Bose (1924) had observed that utilization of malic acid for photosynthesis occurred during the months of April-July when the plants were "acid". In aqueous extracts malic and oxalic acids were detected. He suggested that large amounts of malic acid are formed in darkness and this is utilized as a photosynthetic substrate when the day breaks.

To study whether organic acids are formed due to dark CO_2 fixation *Hydrilla* twigs were allowed to metabolise 100μ C $NaHC^{14}O_3$ in darkness for 15 hrs. One half of the twigs was then immediately extracted in 80 per cent ethanol ; the other half was washed in 10 changes of distilled water and subjected to high intensity light provided by two 1000 watt photoflood lamps at a distance of $1\frac{1}{2}$ feet at 26°C for 4 hrs. and then extracted in ethanol in the usual way.

In darkness only small amounts of $C^{14}O_2$ were fixed. C^{14}-activity was detectable primarily in phosphate esters, and citric-isocitric acid. When the twigs were exposed to light for 4 hrs. radioactivity appeared in malate, sucrose etc.

Utilization of Acetate-1-C^{14} and Acetate-2-C^{14}.

Dark CO_2 fixation, however, may not necessarily make a major contribution to the synthesis of organic acids in darkness in *Hydrilla* and attempts were therefore made to study organic acid metabolism in these tissues after feeding the twigs with C^{14}-labelled acetate which is known to be a key compound in the metabolism of organic acids. Both acetate-1-C^{14} and acetate-2-C^{14} were used.

The experimental twigs were divided into two groups and allowed to metabolise 200μ C acetate-1-C^{14} (sp. activity 37 mg/mC) in darkness for 12 hrs. in small beakers containing 1 ml. M/150 phosphate buffer. At the end of the dark treatment one group was killed by pouring boiling 80 per cent ethanol and the other exposed to direct sunlight for 15 min. at 25°C 80 per cent ethanol extracts after removal of unreacted acetate were plated on stainless steel planchets and counted. A 43 per cent loss of C^{14}-activity was detected in samples exposed to 15 min. light. The distribution of C^{14} in the products of metabolism of acetate-1-C^{14} is given in Table 2. It is clear that while C^{14} activity in several compounds decreases during exposure to light probably due to decarboxylation reactions, it increases sharply in sucrose and starch.

TABLE II

The distribution of C^{14} in the products of acetate-1-C^{14} metabolism in light and darkness in Hydrilla verticillata

Compound	cpm in the extract corresponding to each mg. of tissue (fresh weight)	
	Dark	Light
Sucrose	10,701	35,887
Glutamic acid	65,750	20,090
Aspartic acid	26,100	11,745
Glycine	+	6,655
Citric-Isocitric acid	82,330	13,780
Malic acid	11,745	45,140
Starch*	4,331	23,490

+ traces;   *cpm/mg. of starch.

To demonstrate the actual liberation of $C^{14}O_2$ during exposure to light experiments with 67μC acetate-2-C^{14} (sp. activity 14.9 mg/mC) were performed in Warburg's vessels, the alkali being introduced to the central well immediately before exposure to light. During a 15 min. exposure 17.3 per cent of the total C^{14} activity supplied could be trapped as CO_2 indicating an active decarboxylating process. The products of acetate-2-C^{14} metabolism in darkness and in light are presented in Table 3. The

methyl carbon was found to be metabolised at a rate ¼th that of the carboxyl carbon in darkness. In light the rate increases to ½. In starch in both darkness and light, the methyl carbon is incorporated at a rate twice that of the carboxyl carbon. Sucrose, hexose phosphates and starch considerably increased in light as revealed by the C^{14} incorporation data.

The products of acetate-metabolism in *Hydrilla* are thus rapidly decarboxylated by relatively short exposures to light. A part of the CO_2 liberated, is fixed back into the plant along the normal photosynthetic pathway.

<div align="center">TABLE III</div>

The distribution of C^{14} in certain products of acetate-2-C^{14} metabolism in light and darkness

Compound	cpm in the extract corresponding to each mg. to tissue (fresh weight)	
	Dark	Light
Hexose phosphate	+	59,960
Sucrose	+	1,462
Glutamic acid	+	2,035
Glycine	+	1,722
Unidentified compds. I & II.	39,228	4,489
Starch*	799	3,375

<div align="center">+ traces;   *cpm/mg. of starch.</div>

Utilization of malic acid-2, 3-C^{14}.

In the above experiments with acetate-C^{14} malic acid was found to possess some radioactivity but since a large number of C^{14}-labelled compounds are apparently metabolised at the same time it was not possible to measure the contribution of malic acid to sugar or starch synthesis during the experimental period. Conclusive answers to this question can be obtained in experiments involving the use of malic acid-C^{14}. If carboxyl-labelled malic acid is used an appearance of C^{14}-activity in the carbohydrates would merely mean photosynthetic fixation of CO_2 after decarboxylation but the possibility of the incorporation of carbons 2 and 3 of malic acid in sugar synthesis, cannot necessarily be ruled out since in that case no radioactivity would be detectable in the sugars due to carbons 2 and 3 whic hare non-radioactive. If on the other hand, malic acid-2, 3-C^{14} is employed, an appearance of radioactivity in the sugars or starch may be considered as a contribution of that part of the malic acid molecule which is not susceptible to the decarboxylation reactions. *Hydrilla* twigs were accordingly incubated with $12\mu C$ of malic acid-2, 3-C^{14} (sp. activity 22 mg./mC) in 6 ml. M/180 phosphate buffer of *p*H 6.0 in sunlight at 25°C for 1 min., 5 min. and 60 min. respectively and the C^{14}-labelled compounds analysed as described previously. No radioactivity was observed in sucrose but hexose diphosphate and starch were found to contain bulk of the C^{14} metabolised. It was found that with increase in time C^{14}- activity in starch

increased but that in the sugar phosphates gradually decreased (Fig. 1). Phosphoenol pyruvate was also found to possess C^{14}-activity.
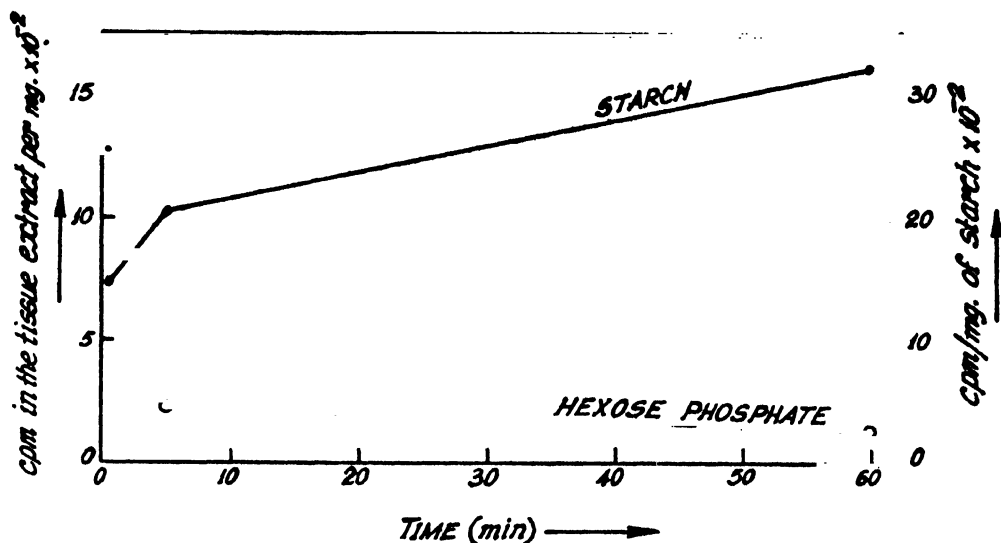
TEXT-FIG. 1.

Incorporation of C^{14} into starch and hexose phosphates from malic acid-2, 3-C^{14} during a 60 minute period.

DISCUSSION

An understanding of the relationship between deacidification and photosynthesis is apparently complicated by the effect of light on respiratory processes in the tissues studied. It is now generally well known that certain amount of CO_2 is evolved when plants are illuminated (Decker 1957; Weigl *et al.* 1951; Krotkov *et al.* 1958; Gyr 1958 ; Soldantov and Ivanova 1955) and the extent of reassimilation depends on the nature of the plant. In the marine flagellate *Dunaliella euchlora* the entire amount of CO_2 liberated is fixed back into the organism by photosynthesis (Ryther 1956). From the observations recorded in the present paper it has been made abundantly clear that *Hydrilla* has a very high rate of CO_2 evolution when illuminated. With acetate-2-C^{14} it has been found that about one-sixth of the total amount of C^{14} -supplied can be recovered as $C^{14}O_2$ during a 15 min. exposure. It should be borne in mind that this CO_2 is the net difference between the fixation and the evolution processes. That the $C^{14}O_2$ evolved was fixed into photosynthetic products was shown by the sharp increase of radioactivity in sucrose as also starch during this 15 min. period. Most of the organic acids present in the plant including malic acid is subject to the decarboxylation process but to what extent malic acid itself was decarboxylated cannot be deciphered from the data on acetate-C^{14} metabolism since malic acid is also known to be produced from CO_2 in relatively short periods of photosynthesis. If, however, the utilization of malic acid in photosynthesis is purely by way of decarboxylation then it could be predicted that when malic acid-2, 3-C^{14}, in which the carboxyl groups are unlabelled is used as a substitute for CO_2 in photosynthesis the sucrose formed would have no radioactivity ; but if other parts of the molecule also contribute to the process the carbohydrates formed would be tagged. It was found

that sucrose itself which is on the CO_2 fixation pathway is non-radioactive but both sugar phosphates and starch were tagged with C^{14}. Kinetic experiments indicated that the specific activity of starch increased with time as activity in the sugar phosphates declined. The only other compound on the chromatograms which was found to possess significant C^{14}-activity was phosphoenol pyruvate. These observations are in agreement with the following concept of malate utilisation in photosynthesis : Malic acid is utilized for photosynthesis in two different ways— (i) malic acid is decarboxylated and the CO_2 liberated is fixed photosynthetically along the conventional pathway associated with the liberation of oxygen which comes from the water molecules participating in the photosynthetic reaction and (ii) the conversion of malate to carbohydrate through phosphoenol pyruvate followed by a complete reversal of glycolysis. The conversion of malate to phosphoenol pyruvate in plant tissues has been demonstrated by Davies (1956). Evidence for the occurrence of the Utter Kurahashi reaction now considered as a major pathway for the conversion of malate to carbohydrate in plant tissues, has come out from the recent studies of Beevers on the conversion of fat to pyruvate (Beevers 1956, 1957 ; Kornberg and Beevers 1957) in the castor bean. That organic acids can be converted into carbohydrates has also been demonstrated by Krotkov *et al* (1954), for tobacco leaves. The mechanism of starch synthesis in *Hydrilla* is not known and on the basis of available data it is not possible at present to speculate on the reaction sequences by which malate is converted to starch without the appearance of any significant activity in sucrose. The latter pathway does not involve any evolution of O_2 ; it is therefore likely that the two processes continue simultaneously.

Malic acid however is not the only organic acid which is metabolised during illumination in *Hydrilla*. Such acids as acetic, fumaric, succinic, citric, α ketoglutaric, aspartic or glutamic are also broken down and 'gas' is evolved. This gas apparently is a mixture of O_2 and CO_2, the CO_2 coming from decarboxylation processes and the O_2 from photosynthesis. The process of decarboxylation under steady state conditions is however very weak as was also found by Kursanov and Krynkova (1957) for *Phaseolus* and the process, thus, is only of limited use for photosynthesis to the plant. Malic acid also is not the major organic acid of *Hydrilla*. The major organic acid has been found to be a substance which moves with the solvent front when extracts of *Hydrilla* twigs are chromatographed in ethanolammonic-water (80: 5: 15). This substance still remains to be identified.

REFERENCES

Beevers, H. (1956). Utilization of glycerol in the tissues of the castor bean seedling. *Plant Physiol.*, **31**, 440–445.
———— (1957). Incorporation of acetate-carbon into sucrose in castor bean tissues. *Biochem J.*, **66**, 23P.
Bose, J. C. (1924). The Physiology of Photosynthesis. Longmans, Green & Co., London.
Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. and Yankwich, P. E. (1949). Isotopic Carbon. John Wiley & Sons., Inc., New York.

Davies, D. D. (1956). Soluble enzymes from pea mitochondria. *J. exp. Bot.*, **7**, 203–218.

Decker, J. P. (1957). Further evidence of increased carbon dioxide production accompanying photosynthesis. *J. Solar Energy Sci. & Engng*, **1**, 30–33.

Gyr, J. (1958). Fixation of CO_2 by leaves of *Pelargonium peltatum* in light and darkness. *C. R. Acad, Sci. Paris*, **246**, 454 (Chem. Abstr. **52**, 9332d).

Kornberg, H. L. and Beevers, H. (1957). The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. *Biochim. biophys. Acta*, **26**, 531–537.

Krotkov, G., Runeckles, V. C. and Thimann, K. V. (1958). Effect of light on the CO_2 absorption and evolution by *Kalanchoe*, wheat and pea leaves. *Plant Physiol.*, **33**, 289–292.

Krotkov, G., Vittorio, P. V. and Reed, G. B. (1954). Synthesis of glucose and starch by tobacco leaves from formic acid-C,[14] acetic acid-1-C^{14}, lactic acid-1-C^{14} lactic acid-1, 2-C^{14} and benzoic acid-$C^{14}OOH$. *Arch. Biochem. Biophys.*, **51**, 147–154.

Kursanov, A. L. and Krynkova, N. N. (1957). The effect of keto and hydroxyacids on the process of photosynthesis. *Biokhem.*, **22**, 391. (*Chem. Abstr.*, **51**, 11486f).

Rabinowitch, E. J. (1945). Photosynthesis and Related Processes. Interscience Publishers, Inc., New York. Vol. I.

Raha, C. R. and Sen, S. P. (1958). Synthesis of DL-malic acid-2, 3-C^{14}. *J. Sci. industr. Res.*, **17B**, 236.

Ryther, J. H. (1956). Interrelation between photosynthesis and respiration in the marine flagellate *Dunaliella euchlora. Nature, Lond.*, **178**, 861–863.

Sen, S. P. and Leopold, A. C. (1956). Influence of light and darkness upon carbon dioxide fixation. *Plant Physiol.*, **31**, 323–329.

Soldatenkov, S. V. and Ivanova, T. P. (1955). The effect of light on the conversion of the organic acids of succulent plants. Uchenye Zapiski Leningrad. Gosudaist Univ. in A. A. Zdhanova No. 186 Ser. Biol. Nauk No. 39, p. 19–38. (*Chem. Abstr.*, **51**, 5926).

Weigl, J. W., Warrington, P. M. and Calvin, M. (1951). The relation of photosynthesis to respiration. *J. Amer. chem. Soc.*, **73**, 5058–5063.

FURTHER OBSERVATIONS ON THE METABOLIC ACTIVITY OF THE SOIL MICROFLORA[1]

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ABSTRACT

The respiratory activity of the microflora of intact, untreated soil has been studied manometrically. Sugars, amino acids, aromatic compounds and tricarboxylic acid cycle intermediates were tested in four soils varying markedly in fertility and organic matter content. The effects of soil reaction and substrate concentration were also investigated. The results are discussed in relation to the use of metabolic or enzymatic activity of soil as an index of fertility.

INTRODUCTION

The use of manometric methods for the study of the metabolic behaviour of soil as an ecological unit has been described previously (Katznelson and Stevenson, 1956). It was found that intact, untreated soil could metabolize mixtures of various substrates such as amino acids, sugars and organic acids. The most striking effect was obtained with casamino acids which appeared to be oxidized adaptively; this adaptation was inhibited by 2, 4-dinitrophenol. Other mixtures of substrates were oxidized directly at a fairly constant rate. Stevenson and Katznelson (1958) have recently reported on the direct oxidation of ethanol and acetate in soil, and studies have been continued in an effort to find other single compounds rather than mixtures, whose oxidation might be used as an index of microbial activity in soils.

The following studies include a general survey of the oxidation of numerous substrates in a number of different soils. In addition the effects of substrate concentration and soil pH on oxidation were investigated.

EXPERIMENTAL

Manometric Techniques

A 4 g. sample of the 0.5–2.0 mm. fraction of a soil was placed in the main chamber of a conventional Warburg vessel. The soil was brought to 60 per cent holding capacity by the direct addition of water or substrate. "Accordion-pleated" pieces of filter paper were inserted in the centre well and 0.2 ml. of 20 per cent, potassium hydroxide added. The vessels were then attached to their respective manometers and placed in the water bath at 30°C. Manometers remained static during the experimental period. Unless otherwise stated results are presented as accumulated oxygen uptake with the appropriate endogenous values subtracted. Inasmuch as soils arriving from the field exhibited considerable variation in moisture content, they were air-dried for a short period before sieving and weighing.

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[1] Contribution No. 480.

[2] National Research Council of Canada Fellow in collaboration with the Canada Department of Agriculture, 1957–1959.

Plating Techniques

Numbers of bacteria and fungi were determined by plating appropriate soil dilutions with Soil Extract Agar and Rose Bengal-Streptomycin Agar.

RESULTS

Initial studies were undertaken with a variety of substrates in different concentration in one soil. Accumulative oxygen uptake values for a period of six hours are given in Table 1.

TABLE I

Oxidation of a Variety of Substrates in Soil

Substrate	Conc.* mgms.	O_2 uptake	Substrate	Conc.* μM	O_2 uptake
Sucrose	5	288	Na Benzoate	25	66A
	10	389		50	22A
	20	252	Catechol	25	19A
Lactose	5	13A**		50	42A
	10	33A	Guiacol	25	0
	20	6A		50	0
Maltose	5	89	Vanillin	25	13A
	10	123		50	0A
	20	74	l-Alanine	25	3
Fructose	5	72		50	17
	10	41	Arginine	25	27
	20	65		50	73
Glucose	5	133	Glycine	25	58
	10	124		50	59
	20	89			
Galactose	5	0	Na Pyruvate	25	50
	10	0	Na Citrate	25	20A
	20	20	α-ketoglutarate	25	20A
Xylose	5	0	Na Succinate	25	158
	10	6A	Na Fumarate	25	120
	20	19A	Na Malate	25	141
Arabinose	5	12	Na Acetate	25	30
	10	23	Na Cis-Aconitate	25	28
	20	19	Na Iso-citrate	25	0

*Concentrations of substrates per 4.0 gms. soil.
**Adaptive oxidation.

Sucrose proved to be the most readily oxidizable substrate used and it is noteworthy that the oxygen uptake for this compound was greater than the sum of the oxygen values for fructose and glucose in equivalent concentrations. Maltose was utilized at a moderate rate whereas lactose oxidation was very slow. Glucose was the most readily available of the three hexose sugars tested while little activity was noted with galactose. Pentose sugars were also oxidized very slowly with a slow adaptation observed in the case of xylose. With the amino acids studied, glycine was found to be oxidized at a steady rate while the oxidation of l-alanine and arginine proceeded more slowly. Little oxygen uptake was noted with the aromatic compounds and this usually occurred after a fairly long lag period varying from two hours for benzoate and catechol to four hours for vanillin. A distinct inhibition of respiration occurred with guiacol and with the higher concentration of benzoate and vanillin. Of the tricarboxylic acid cycle intermediates the dicarboxylic acids were oxidized most rapidly and directly. Pyruvate and acetate were

utilized more slowly.   The remaining acids were oxidized very slowly with a gradual adaptation occurring.

A comparison of the oxidation of seven selected substrates by three other soils of varying fertility and organic matter contents are presented in Figure 1.
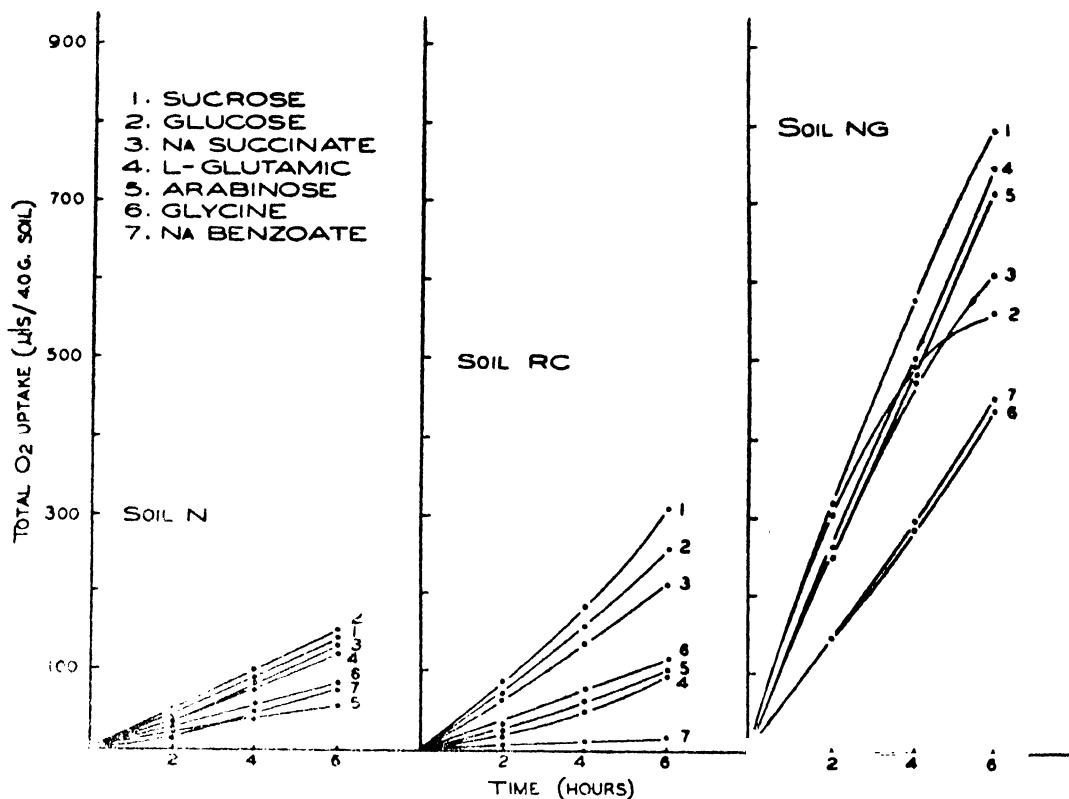
TEXT-FIG. 1

Oxidation of seven selected substrates in three soils. Warburg vessels contained 4.0 gms. of soil supplemented with 25μ Ms of substrate.

A distinct gradation in overall activity is observed among the three soils illustrated.   All substrates are metabolized extremely rapidly in soil NG.   This soil is the highest in organic matter content (Stevenson, 1956) as well as in metabolic activity (Katznelson and Stevenson, 1956).   Intermediary rates of oxidation are noted in the case of the Rideau Clay (RC) soil whereas least activity is observed in the relatively infertile soil N.   It is of interest to note that the substrates most actively oxidized by soil X (Table 1), namely, sucrose, glucose and succinate are also oxidized most readily by soils N and RC.   Rates of oxidation of these substrates are also among the highest with soil NG although substrate concentrations may have become limiting in the case of succinate and glucose.

The effect of substrate concentration on the oxidation of succinate in soil NG is illustrated in Fig. 2.

With extremely low concentration (5-10μ M) the initial oxidation rates are somewhat lower than those of the higher concentrations.   In all cases the duration of this intial direct oxidation increases as substrate concentration increases, to be followed by a rapid decline in oxidation rates.

In order to study the effect of pH on substrate oxidation soil NG was treated with varying amounts of HCl to provide a series of samples of pH 3.2, 3.7, 4.5, 5.5 and 6.5. The oxidation data for sucrose, succinate and l-glutamic acid in these acidified soils are presented in Figure 3.
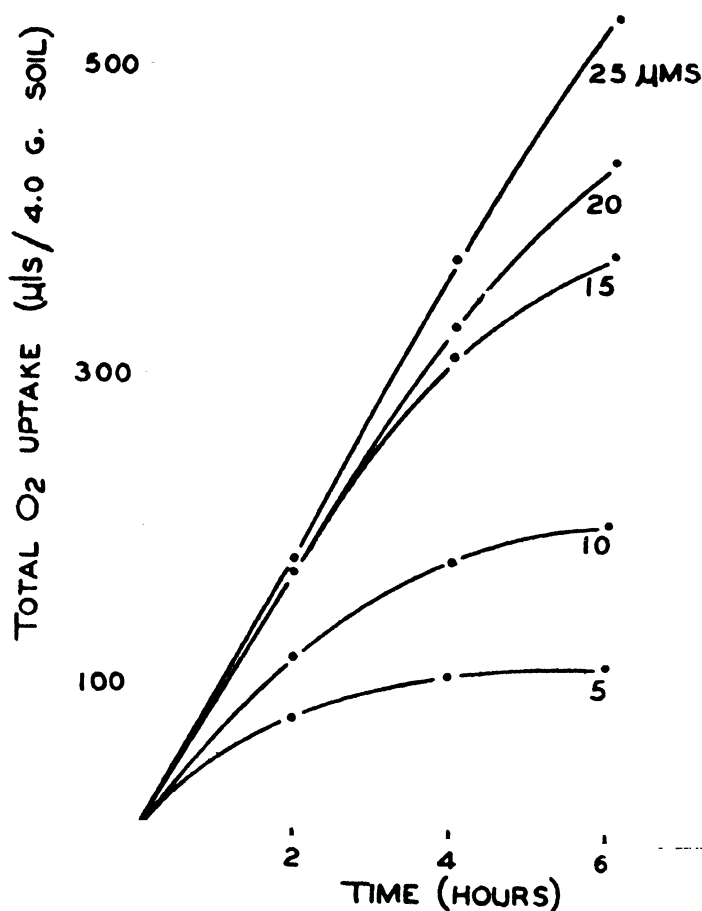
TEXT-FIG. 2

The effect of different concentrations of succinate on oxidation in soil NG.

A marked decrease in activity is noted with all substrates as the soil pH is lowered though some oxidation is still evident even at pH 3.2. Numbers of bacteria and fungi were also determined in acidified soils after a 6 hour incubation period. These data are given in Table II.
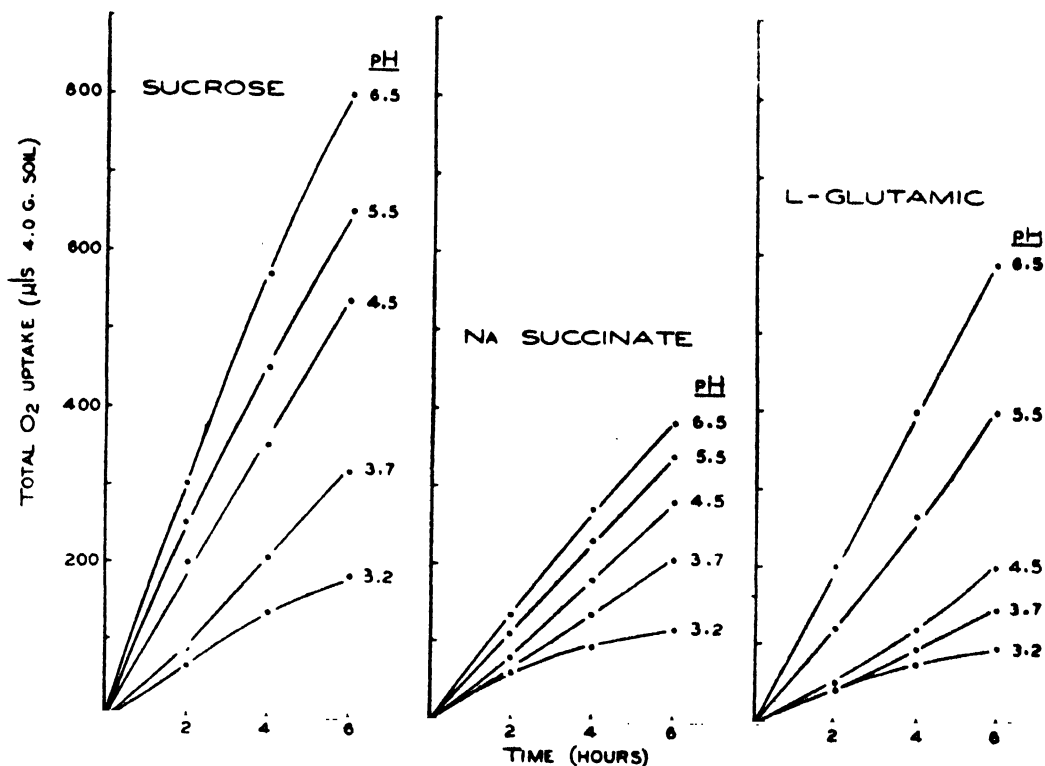
TEXT-FIG. 3

The oxidation of sucrose, succinate and l-glutamic acid in soil NG at different pH's.
Soil supplemented with 25μ Ms substrate.

TABLE II

Number of Bacteria and Fungi in Soil at Different pH's*

Soil	Bacteria** $\times 10^6$	Fungi*** $\times 10^6$
Control Unadjusted	33.0	13.6
pH 6.5	35.6	14.0
pH 5.5	32.0	29.6
pH 4.5	20.3	11.0
pH 3.2	6.3	8.0

*Soils adjusted to appropriate pH and allowed to stand 6 hours prior
 to plating.
**Plate count in Soil Extract agar.
***Plate count in Rose Bengal-Streptomycin agar.

It is evident that the reduction of respiration with increasing acidity occurs
before a noticeable reduction in plate counts takes place. Below pH 5.5 there

is a significant drop in bacteria and this is especially noticeable at pH 3.2. Numbers of fungi do not decrease to the same extent.

The rapid oxidation of sucrose in the various soils suggested further investigation as to its breakdown products. Twenty gram samples of soil NG were brought to 60 per cent water holding capacity with a 1 per cent sucrose solution so that the final concentration was equal to 10μ M sucrose per gram of soil. Samples were incubated at 30°C and extracted with water at intervals of 2, 5, 24 and 48 hours. Aliquots of the aqueous extracts were spotted on filter paper and chromatographed with n-butanol : acetic acid : water (4 : 1 : 5); the papers were sprayed with solutions of silver nitrate or 2,-aminobiphenyl. Little, if any, hexose sugars could be detected at the 2 and 5 hour extraction periods though sucrose was still present in relatively high concentrations. By 24 hours glucose and fructose spots were distinct while the concentration of sucrose had decreased. At 48 hours no evidence of sucrose was found whereas traces of the hexose sugars were still evident. It is of interest to note that these data like the respiration data show the rapid disappearance of sucrose with a slower oxidation of the constituent hexoses.

DISCUSSION

The pattern of substrate oxidation by the four soils used in this study in which sucrose, glucose and succinate were favoured may be of general significance in relation to the metabolic activity of the soil microflora. Sucrose and glucose occur in both the free and combined state in plant residues and are readily attacked by a wide variety of soil micro organisms. Succinate is a common respiratory intermediate and it is not inconceivable that soils would contain a microflora well adapted to utilize this compound. Sugars such as lactose or galactose not usually found in plant debris are oxidized very slowly or after adaptation as in the case of lactose (Table 1). Pentoses are not usually present as free sugars in plant material and are only liberated through hydrolysis of more complex molecules such as gums, hemicelluloses and nucleic acids (Bonner, 1950). It might be expected therefore that the soil microflora would not be adapted to utilize these substrates directly. It is of interest to note that the highly organic soil NG appears to contain a population which is able to oxidize arabinose very rapidly.

Aromatic substances are not found in abundance in soil. They originate from the slow decomposition of substances such as lignins and do not accumulate to any extent. In consequence a relatively small population is present capable of direct oxidation although the soil population appears to be able to adapt itself slowly to these compounds.

The oxidation of amino acids varies considerably with the different soils. Since these compounds are liberated regularly from plants and plant constituents it was expected that the soil population would utilize them as rapidly as they were produced. Oxygen uptake values for these subtrates were found to be relatively low but in view of the numerous non-oxidative conversions amino acids can undergo this is not surprising.

The intermediates of the tricarboxylic acid cycle are widely distributed in plants and appear readily available to soil micro-organisms. Four-carbon acids such as succinic, fumaric and malic acids are the most rapidly oxidized. Acids such as citric, iso-citric and cis-aconitic are not readily oxidizable ; this may well be due to permeability barriers.

An attempt was also made during these studies to determine the groups of soil organisms concerned in the overall metabolic activity observed. Experiments with selective antibiotics such as actidione and streptomycin or chloromycetin gave inconclusive results (unpublished data). The antifungal agent (actidione) did not reduce soil respiration in the presence of substrate whereas the antibacterial agents caused only partial reduction in activity; however, lowering the pH of the soil

caused a consistent and marked decrease in oxygen uptake with the three substrates used. There was no correlation between numbers of bacteria and fungi and respiration between the pH range of 5.5 and 6.5 and it may be expected that the decrease in respiration was due to a general inhibition of the enzymes concerned. At pH 4.5 and especially at pH 3.2 there was a marked reduction in numbers of bacteria. The fungal count was less severely affected by the acid environment though at pH 3.2 there was a forty per cent reduction in numbers. The reduction of both bacteria and fungi at the lowest pH values may account for the low metabolic activity of the soils with emphasis on the severe reduction in bacterial numbers. The possibility of a direct or indirect pH effect on other types of soil organisms, such as protozoa, can not be disregarded, however.

A number of investigators have studied invertase activity in soil and have attempted to relate the results of overall microbial activity and soil fertility (Kiss, 1957; Seegerer, 1953). The data obtained in the present investigations through respiratory and chromatographic studies support the reports of active invertase in soils. Variation in activity in different soils as indicated in Fig. 2 suggests the possibility of using 'invertase activity' as a criterion for assessing microbial activity or fertility of soils.

REFERENCES

Bonner, J. (1950). Plant Biochemistry. Academic Press Inc., New York.
Katznelson, H. and Stevenson, I. L. (1956). Observations on the metabolic activity of the soil microflora. *Canad. J. Microbiol.*, **2**, 611–622.
Kiss, S. (1957). Die Wirkung des spezifischen Enzymsubstrates (Saccharose) auf die Produktion der Bodensaccharase. *Z. PflErnähr. Düng.*, **76**, 119–122.
Seegerer, A. (1953). Der Saccharasgehalt des Bodens als Massstab seiner biologischen Activität. *Ibid.*, **61**, 251–260.
Stevenson, I. L. (1956). Some observations on the microbial activity in remoistened air-dried soils. *Plant & Soil*, **8**, 170–182.
Stevenson, I. L. and Katznelson, H. (1958). The oxidation of ethanol and acetate in soils. *Canad. J. Microbiol.*, **4**, 73–79.

ON A FEW FOSSIL SHARK TEETH FROM THE MIOCENE BEDS OF KUTCH, WESTERN INDIA

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(Communicated by A. G. Jhingran, F.N.I.)

ABSTRACT

Carchariolamna heroni Hora and *Hypoprion horai* sp. nov., fossil shark teeth, have been described from the Lower Miocene beds of South-Western Kutch, India. These finds throw light on the age of Balasore and Baripada beds and do not favour the view of Eocene age for these beds.

INTRODUCTION

The present paper deals with a few fossil shark teeth assigned to the genus *Carchariolamna* Hora 1939 and *Hypoprion* Müller and Henle. These have been isolated from the foraminiferal marls occurring in the neighbourhood of the villages Vinjhan (N. 27° 6' : E. 69° 2') and Waior (N. 23° 25' : E. 68° 44'), south-western Kutch. The Marls are highly fossiliferous, and almost entirely made up of foraminifers, ostracoods and other invertebrate fossils, which are being studied in detail by the author. These are undoubtedly of Lower Miocene age (Gaj Series), as concluded from the association of foraminifers and molluscs. The material was collected by the author in the months of September and October, 1949 and later on supplimented by further collection in the winter of 1952.
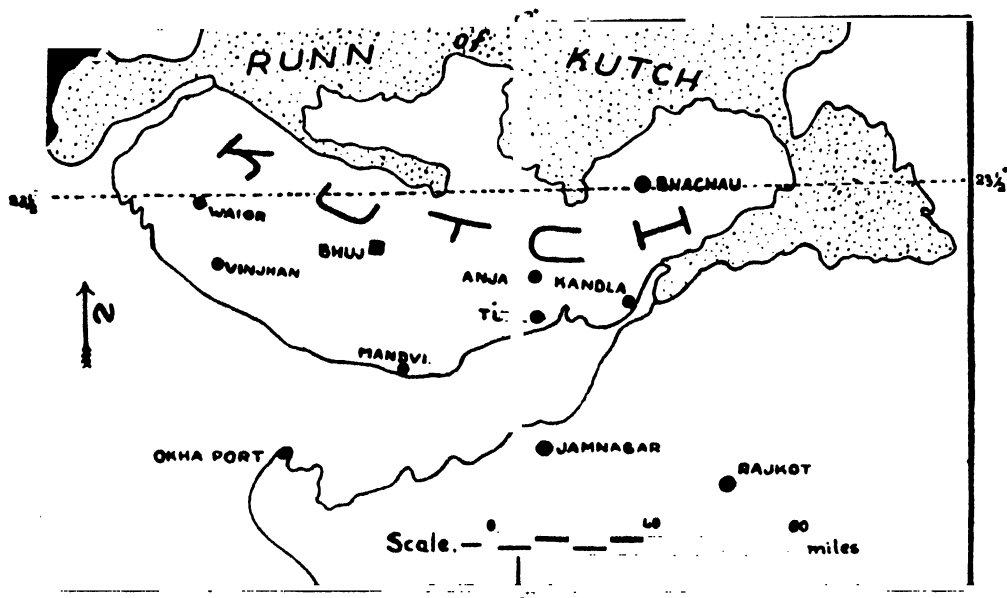
TEXT-FIG. 1.

At Vinjhan (Tewari, 1952) the foraminiferal marl is about 50 feet thick, yellow in colour with abundant *Taberina malabarica* (Carter). On this basis this horizon has been called the zone of *Taberina malabarica*. It dips at about 5° towards

S.S.W. and is overlain by a cream-coloured compact limestone full of *Miogypsina* s.s. and underlain by a zone containing *Corbula* and *Turritella*.

At Waior this marl is about 12 feet thick and has a yellow brown colour. It is underlain by a band of limestone full of *Operculina* together with *Miogypsina* and *Miogypsinoides* and itself overlain by loose coarse-grained sandstones and dips at a very low angle of 3° to 5° towards south. This section of rocks can be followed down-stream in the rivulet passing through the villages of Waior and Waghot (N. 23° 24′ : E. 68° 44′).

The shark teeth, which form the subject matter of this paper, are the only vertebrate fossils, which have been so far observed by the author, in these beds and hence are of particular interest. Furthermore, the occurrence of these fossils, in the undoubtedly Lower Miocene beds of Kutch, has a bearing on the age and correlation of the Balasore and Baripada beds of Orissa, India.

PREVIOUS WORK

Apart from the researches of Lydekker (1886) on the fossil fishes from the Siwalik rocks of India, Noetling as long ago as 1901 called attention to the occurrence of shark teeth from the Miocene beds of Burma. A little later, Murray Stuart (1909-10) reported and illustrated fossil fish teeth collected from the Pegu System of Burma. Some years later Gee (1926) obtained from the Tertiary beds of Andaman Islands several types of fishes' teeth, which he considered to belong to sharks. The remains of fossil fishes from India and Pakistan and problems associated with their occurrence have been studied by Hora (1936, 1937a, b, c d, 1938a, b, 1953) in detail. He has also contributed an excellent paper on the fossil fish-remains from Balasore, Orissa (Hora, 1939) wherein he has described several fossil teeth, out of which teeth of *Carchariolamna heroni* Hora 1939 and *Hypoprion* are of particular interest to us. More recently Misra (1947 and 1951) has published immensely useful paper especially a 'Check List' and 'Key' for the identification of fishes of India and adjacent countries. Similarly Menon (1951) has also dealt with the distribution of fishes in the past and their bearing on the palaeogeography of India. Tewari (1954) has reported *Carchariolamna heroni* Hora from the Lower Miocene beds of Kutch. Besides these Rao (1956) has made a study of a fossil Siluriod fish from the Eocene rocks of Western Kutch. The principal characters of *Carchariolamna* (Hora, 1939, p. 202) are described as :

> "It bears close affinity with the Eocene Genus *Carcharoides* from which it is distinguished by the less developed or totally absent lateral denticles, very finely serrated edges, and an almost erect and blunt crown.

The teeth are solid throughout and are, therefore, referable to the family Lamnidae and not to the Carcharinidae, in which the teeth are invariably hollow."

DESCRIPTION OF SPECIES

Class—ELASMOBRANCHII
Sub-class—SELACHII
Order—EUSELACHII
Family—LAMNIDAE
Genus—*Carchariolamna* Hora 1939

Charchariolamna heroni Hora 1939
Pl. X, figs. 1-3 ; Text-fig. 2, fig. 3.

Carchariolamna heroni Hora, 1939, *Rec. Geol. Surv. India*, 74 (2) 203-205, pl. 13, figs. 1-4, Text-fig. 1b.

Carchariolamna heroni Hora Tewari, 1954, *Proc. Ind. Sci. Cong. 41* (4), p. 14.

The present tooth is well preserved except for a small portion of its base. The surface of the tooth is shining and it is quite stout and consists of a crown or cusp which is strong and straight. Its apex is blunt and the margins are thin, compressed and finely serrated. The serrations are not present on the base of the crown but they almost extend to the top and bottom of the crown. The external side of the crown is convex and its surface is almost flat. The internal side is concave and its surface is slightly convex.

The base is broad, strong and slightly arched. There is a median groove, extending from the bottom to almost the top, on the internal surface of the base.

A small groove is also seen at the junction of the crown and the base, which is about 0.25 mm. wide. The lateral denticles are incipient or almost absent.

LOCALITY

The single tooth has been obtained from the Lower Miocene (Burdigalian) beds of Kutch. The specimen comes from the zone of *Taberina malabarica* two furlongs north-east of the village Vinjhan (N. 27° 6′ : E. 69° 2′) in southeastern Kutch.

ASSOCIATION

It has been found associated with *Taberina malabarica* (Carter). *Miogypsina* (*Miogypsina*) *irregularis* Michelotti, *M.* (*Lepidosemicyclina*) *indonesiensis* Tan, *Austrotrillina howchini* (Schlumberger), *Miogypsina* (*Miolepidocyclina*) *burdigalensis* (Gümbel), *Sorites marginalis* (Lamarck), *Archaias angulatus* (Fich. & Moll.), *Streblus. Ostrea latimarginata* Vredenburg, *Ostrea* (*Lopha*) *virleti* Deshayes, *Ostrea digitata* Eichwald, *Turritella* (*Torculoidella*) *angulata* (Sowerby), *Clypeaster waageni* Duncan and Sladen, *Spondylus waylandi* Davies, *Bairdia subdeltoidea*, and a host of smaller foraminifers and ostracods.

REMARKS

The present tooth is smaller than the one described by Hora from Balasore, but it resembles very much in shape and other essential characters to the type species. There are minor differences between the present tooth and the holotype of the type species, but it resembles most closely the co-type no. 16649 (Hora 1941, plate 13, fig. 4) kept in the Geological Survey of Indian Museum. The extraordinarily small size of the tooth is possibly due to its being young specimen, and it may, therefore, be safely concluded that the present tooth belongs to *Carchariolamna heroni* Hora.

Family CARCHARINIDAE
Genus *Hypoprion* Müller & Henle

Hypoprion horai sp. nov.
Pl. X, figs. 4, 5 ; Text-fig. 2, figs. 1, 2.

Hypoprion Müller & Henle, Hora, 1939, *Rec. Geol. Surv. India*, 74, pt. 2, 208-209, Text-fig. 5a.

For the present study three well preserved teeth were isolated from the foraminiferal yellow brown marl of Waior. These are small and triangular with an oblique, pointed and hallow crown, the edges of which are thin, compressed and smooth except at the bases, where they are coarsely serrated on one side and crenulated on the other. These have a shining surface, and are of amber colour.

1

2

3

4

5

Fig. 1. *Carchariolamna heroni*. View of the external side of tooth showing denticulations on the corwn and its flat external surface. Vinjhan, Kutch. ×5½.

Fig. 2. *Carchariolamna heroni*. View of the internal side of the tooth showing its convex internal surface of the crown and groove on the base. Vinjhan, Kutch. ×5½.

Fig. 3. *Carchariolamna heroni*. Lateral view of the tooth. Vinjhan, Kutch. ×3½.

Figs. 4 & 5. *Hypoprion horai* sp. nov. View of the internal side of the teeth showing serrations at the base of the crown and the groove at the base. Waior, Kutch. ×5½.

The external surface of the crown is flat and the internal one is convex. The moderately arched base of the tooth is broad and strong. There is a median groove on the internal surface extending from the bottom to almost the top.
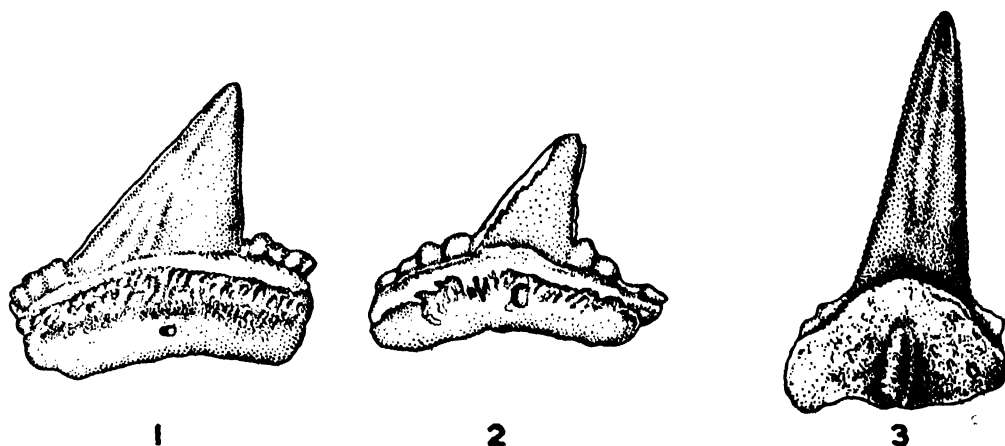
TEXT-FIG. 2.

The present species has been named in the honour of late Dr. S. L. Hora, who first discovered it from the Balasore and Baripada beds, which have been assigned an Upper Tertiary age.

LOCALITY

The specimens have been collected from about 2 furlongs south of Waior (N. 23° 25′ : E. 68° 44′). They come from a yellowish brown marl assigned to the basal part of Lower Miocene.

ASSOCIATION

These have been found occurring in association with *Operculina, Spiroclypeus* sp. indt. *Miogypsina (M.) irregularis* (Michelotti), *Austrotrillina howchini* (Schlumberger), *L. (Nephrolepidina) sumatrensis* (Brady), *L. (N.) börneensis* (Provale), *(M.) Miogypsinoides dehaarti* Vander Vlerk, *Turritella (Torculoidella) angulata* (Sowerby), fossil algae and ostracods.

REMARKS

The present teeth from Kutch are remarkably similar in shape and structure to that described by Hora from Balasore. However, minor variations amongst the teeth of the present species have been observed, which may be due to their different positions in the jaws.

CONCLUSION

The beds containing *Taberina malabarica, Spondylus waylandi* and *Clypeaster carteri* from Ceylon were assigned to Vindobonian by Wayland and Davies (1923). Later Eames (1950), after a detailed study of the molluscan fauna of the Miocene of Ceylon, Quilon, Karikal, Cuddalore, Kathiawar and Kutch, concluded that the beds containing the above cannot be younger than Burdigalian (Upper Gaj). The

MEASUREMENTS

Name and Locality	Total Length	Height of crown	Height of base	Maximum width of crown base	Thickness of crown	Height of base at outer edge	Maximum width of base	Maximum thickness of base
Carchariolamna heroni Balasore	18 mm.	14.4 mm.	3.6 mm.	8.5 mm.	3.9 mm.	Approx. 4.5 mm.		
Carchariolamna heroni Kutch	6.5 mm.	4.2 mm.	2.3 mm.	2.0 mm.	1.5 mm.	1.5 mm.	4.0 mm.	1.8 mm.
Hypoprion horai I Kutch	3.7 mm.	2.1 mm.	1.6 mm.	1.6 mm.	0.8 mm.	1.2 mm.	5.2 mm.	1.1 mm.
II	5.0 mm.	3.0 mm.	2.0 mm.	3.0 mm.	1.1 mm.	1.5 mm.	5.9 mm.	1.5 mm.

evidences, of foraminifers such as *Austrotrillina howchini* and of molluscs like *Ostrea latimarginata* found associated with *Carchariolamna heroni* and *Taberina malabarica* in Kutch are in favour of these beds being included in the Upper part of the Lower Miocene (Burdigalian).

Similarly the foraminiferal fauna found associated with *Hypoprion horai* is characteristic of the basal part of the Lower Miocene (Aquitanian). This has been concluded from the presence of *Miogypsina* s.l. together with *Spiroclypeus* (Glaessner 1951 and Eames 1953).

Therefore, it follows from these considerations that the present occurrence of *Carchariolamna heroni* and *Hypoprion horai* in beds of undoubtedly Lower Miocene age of Kutch is an additional evidence in favour of the Balasore and Baripada beds being of Upper Tertiary age (Tewari 1954). However, these occurrences alone are quite inadequate for the correlation of the Balasore and Baripada beds with those of Lower Miocene beds of Kutch, but the available evidence as also indicated by Sahni (Hora 1941) is definitely in favour of these beds of Upper Tertiary age, and most certainly contradict the Eocene age for these beds.

ACKNOWLEDGEMENTS

REFERENCES

Eames, F. E. (1953). The Miocene/Oligocene boundary and the use of the term Aquitanian. *Geol. Mag.*, **90**(6), 388-392.

Gee, E. R. (1926). Geology of Andaman and Nicobar Islands. *Rec. geol. Surv. India*, **59**(2), 208-232.

Glaessner, M. F. (1951). Three foraminiferal zones in the Tertiary of Australia. *Geol. Mag.*, **88**, 273-282.

Hora, S. L. (1936). The fish of Chitral. *Rec. Indian Mus.*, **34**, 307-310.

———— (1937a). Fossil fish-remains from the Saline Series of North-Western India. *Rec. geol. Surv. India*, **72**(2), 188-194, pl. 15, figs. 1-6.

———— (1937b). On fossil fish remains from Karewas of Kashmere. *Ibid.*, **72**(2), 178-187 pl. 14, figs. 1-6.

———— (1937c). On a shark tooth from the Lower Eocene. *Ibid.*, **72**(2), 174-177, text figs. 1 a-c.

———— (1937d). Distribution of Himalayan fishes and its bearing and certain palaeogeographical problems. *Rec. Indian Mus.*, **39**, 251-259.

———— (1938a). On some fossil fish scales from the Intertrappean beds of Deothan and Kheri, C.P., *Rec. geol. Surv. India*, **73**(2), 267-294, pls. 17-18.

———— (1938b). On the age of the Deccan Trap as evidenced by fossil fish remains. *Curr. Sci.*, **6**, 370-372.

———— (1939). On two small collections of fossil fish remains from Balasore, Orissa. *Ibid.*, **74**(2), 119-215.

———— (1953). An Ichthyologist looks at Indian Palaeogeography. Anniversary Address, Nat Inst. Sci. India, 1-13.

Lydekker, R. (1886). Indian Tertiary and Post-Tertiary Vertebrata: Tertiary fishes. *Palaeont. Indica, Ser.* 10, **3**(8)

Menon, A. G. K. (1951). Further studies regarding Hora's Satpura Hypothesis. (1) The rôle of Eastern Ghats in the distribution of Malayan fauna and flora to peninsular India. *Proc. nat. Inst. Sci. India*, **17**, 475-497.

Misra, K. S. (1947). 'A Check List' of the fishes of India, Burma and Ceylon. Pt. I. Elasmo-branchii. *Rec. Indian Mus.*, **45**(1), 1-46.

———— (1951). An aid to the identification of the fishes of India, Burma and Ceylon. I. Elasmobranchii and Holocephalii. *Ibid.*, **49**(1), 89-137.

Murray Stuart (1909). Fossil fish teeth from the Pegu System of Burma. *Rec. geol. Surv. India*, **38**(4), 292-301

Rao, V. R. (1956).   The skull of an Eocene Siluroid fish from Western Kutch, India.   *Palaeont. Soc. India*, Inaugural Number, 181–185.

Tewari, B. S. (1952).   The Tertiary beds of Vinjhan-Miani area, southeastern Kutch, India. *Curr. Sci.* **21**. 217–218.

———— (1954).   On a fossil shark tooth from the Miocene beds of Kutch, India.   *Proc. Indian Sci. Congr.*, **41**(4), 14.

Wayland, E. J. and Davis, A. M. (1923).   The Miocene of Ceylon.   *Quart. J. geol. Soc.*, **79**(4), 577–602.

A STATISTICAL STUDY OF GROWTH IN PARTS OF THE SECOND PAIR OF CHELIPEDS IN SOME SPECIES OF THE INDIAN FRESHWATER PRAWNS OF THE GENUS *PALAEMON*

by R. K. MISRA, *University of Saugar*

(Communicated by W. D. West, F.N.I.)

ABSTRACT

◆

In the present paper growth in the second pair of chelipeds and their component parts, the Is., Me., Ca., Pro. and the Dac., has been studied in the light of the growth formula,

$$y = bx^{(a+ax)}e^{cx}$$

in properly collected samples of the male and the female Indian freshwater prawns of the following four species of *Palaemon* Fabr. :
     (i) *Palaemon* dayanus Henderson.
     (ii) *Palaemon* lamarrei H. Milne—Edwards.
     (iii) *Palaemon* Kistnensis Tiwari, and
     (iv) *Palaemon* hendersoni DeMan.
The following points emerged from this study :

1. It is seen that there is only one phase of growth in the male and the female chelipeds in each of the three species, other than P. hendersoni, with a progressive change in the values of the growth-coefficient. The values of the equilibrium constant a coincide with the mean p within the limits of sampling fluctuations.

2. In P. dayanus the phase of growth in the males starts with a growth-centre in the Pro. and a point of depression in the Me. which develops into a growth-centre towards the end. In the female organ it starts with a growth-centre in the Me. and a point of depression in the Dac. which later develops into a steep high point. The growth-gradient in the male cheliped, in P. lamarrei, starts with a growth-centre in the Ca. and, as in the males of P. dayanus, a point of depression in the Me. which develops into a growth-centre towards the end of the phase; while in the female organ of this species there is no change in the growth-gradient pattern throughout the phase, thus showing here a stable state of growth. Pro. forms a growth-centre here. In P. Kistnensis growth in the male cheliped starts with a growth-centre in the Me. which becomes a point of depression between the neighbouring joints, the Is. and the Ca., towards the end of the phase, when the Pro. forms a growth-centre here, and remains as the only positively heterogonic joint. In the female organ the Me., which is a point of depression at the start, developes into a growth-centre towards the end of the phase; while the Dac. forms a high point throughout it.

3. In P. dayanus the gradient in the female cheliped seems to be much more marked than the one in the male. In P. lamarrei, however, the gradient in the female cheliped remains perfectly unchanged for all lengths of the cheliped, whereas in the male cheliped it is a changing one and is associated with a rapid shift of the growth-centre from the central (Ca.) to the basal (Me.) region of the organ. In P. Kistnensis the gradient in the male cheliped is highly marked (and is associated with a rapid shift of the growth-centre from the Me. of the basal region to the Pro. of the distal region); whereas in the female cheliped the gradient is very stable and is associated with only a slow development of heterogony in the Me. and a progressive and gradual change in the values of the relative growth rates of the other segments of the organ. In P. hendersoni the gradient in the male cheliped (I Phase) is more marked than the one in the female. In fact in the male there takes place a rapid progress in the values of the relative growth rates in the segments of the distal region of the organ, a steep fall from a positive to a negative heterogony in the central region and a development of the growth-centre in the Me. of the basal region. The second phase of growth in the male cheliped, however, forms a highly marked growth-gradient progressing in one direction only and resulting in the progress from , less to a more steep growth-centre at the Pro. of the distal region and a development of another, but less marked, growth-centre at the Me. of the basal region.

INTRODUCTION

For a very long time the study of differential growth in an organism had received little attention. The idea of growth-gradient in an organic form was for the first time beautifully put forth by D'Arcy Thompson (1917, 1942); but his studies, though fascinating and original in many respects (e.g. his use of cartesian transformations in studying the evolution of one form from another), lacked the quantitative expression that *prima facie* seems to govern the process of growth in the animal kingdom.

A new impetus and interpretation to the study of differential growth was given by Huxley (1932). He has analysed a vast body of data on animals and plants and has arrived at certain quantitative deductions about the laws that seem to govern the growth of organic life in general; and, although originated by Przibram (1902, 1917), Champy (1922, 1942) and others, the formula for allometric growth, known as the simple allometry equation, was given a new status by him (*op. cit.*) in studying the growth process in a living organism. It is,

$$y = bx^a,$$

where x and y represent respectively the sizes of the body and the organ; b and α are two constants of which the latter, known as the 'equilibrium constant' or the 'growth-coefficient', is of particular interest.

It was next seen that although the simple allometry equation has proved of very wide importance in understanding the dynamics of a body, still certain phenomena of growth could not be explained clearly because it has some shortcomings, some of which have been discussed by Huxley himself (*op. cit.*). In this connection the author of the present paper has already established (1957) that this allometric equation is only a valuable first approximation of the following more general formula of growth,

$$y = bx^{(a+ax)}e^{cx} \qquad \qquad .. \quad (1)$$

where b, α, a and c are constants and e stands for the exponential; and that the equilibrium constant α should be replaced by ρ where,

$$\rho = \alpha + (a + c)\, x + ax \, \log_e x \qquad \qquad .. \quad (2)$$

GROWTH IN INDIAN FRESHWATER PRAWNS OF THE GENUS PALAEMON

Besides summarising a large body of data from animal and plant kingdoms Huxley (*op. cit.*) has also indicated the various fields in biology which require intensive investigations. One such field is Taxonomy. For the purpose of systematic Zoology where body proportions often play important role in delimitation of species, investigation on relative growth of parts in relation to the rest of the body, or in relation to each other, can throw much light on the true taxonomic status of species. The precise mathematical treatment easily defines the limits of species and thus eliminates a lot of confusion about dubious characters, which otherwise often arises and results in the false establishment of new species.

Decapoda crustacea have always been very illustrative example for relative growth. The genus *Palaemon* is a decapod crustacea. It occurs mostly in the freshwaters of the tropics and sub-tropics. It belongs to the group Macrura or the prawn with a long abdomen. In India there are over three dozen species of this genus showing all sizes of body and appendages. One striking feature in most of these species is the conspicuously large growth that the second pair of chelipeds attain after some age.

With respect to the size of the second pair of chelipeds Indian species can be easily divided into three groups, viz., those with slender chelipeds which are much shorter than the body, those with shorter but stouter chelipeds and those inwhich these appendages are very long and strongly built. While some species belonging to the last group have been subjected to researches on relative growth (Huxley, *op. cit.*, Tazelaar, 1930, etc.), no work has been done on species of other groups.

The author of the present paper has taken for study the following four species of *Palaemon* Fabr. of the other groups :

   (i) *Palaemon* dayanus Henderson (87 male and 66 female specimens),

   (ii) *Palaemon* lamarrei H. Milne-Edwards (74 male and 80 female specimens),

   (iii) *Palaemon* Kistnensis Tiwari (53 male and 43 female specimens), and

   (iv) *Palaemon* hendersoni DeMan (90 male and 69 female specimens), some discussion on the last one of which has already been done by the author (1957, 1958).

These represent two types, each type having certain specific growth behaviour which is manifested in the differences in the growth ratios of the whole limbs and their component constituents. Of these P. dayanus and P. lamarrei are very widely distributed throughout the Indian plains and a major part of the peninsula, while the other two have restricted distribution.

MATERIAL AND MEASUREMENT

The studies here have been solely confined to the growth behaviour of the, second pair of chelipeds, in the light of the above formula (1) for growth, in males and females of the species named here. The Director, Zoological Survey of India has done me the favour of providing with the material which is now kept in the reserve collections of the Z.S.I.

Sliding chelipers fitted with graduated dial which could read directly upto the first place of decimals were used to measure length (in millimetres) in the following way :

   (i) Length of the carapace was measured in a straight line between the orbital edge and the posterior border.

   (ii) Lengths of the segments of the cheliped were measured dorsally in a single straight line, and

   (iii) The total length of the cheliped (for which the abbreviation Ch. will be used henceforth) was calculated by adding up the lengths of the Ischium, Merus, Carpus, Propodus and the Dactylus (abbreviations : Is., Me., Ca., Pro. and Dac. respectively). In a cheliped there are seven such segments, but the first two. viz., coxa and basis are firmly fused with the Is. and are of negligible length.

Further, as some extraneous factor like autotomy and regeneration alone could possibly be responsible for the outward difference in the lengths of the dextral and sinistral second chelipeds, which should basically have no distinction in their growth rates, the length of the larger of the two chelipeds only has been used in the data.

OBSERVATIONS AND CALCULATIONS

The values of the log carapace length (log stands for the logarithm), denoted as X, and of log length of the cheliped or its segments, denoted as Y, have been grouped for X, as shown in Table 1 which gives for each group the average of the values of X and Y lying in it.

TABLE I

Showing the values of the group averages for X and Y

Group	No. of specimens	Average X	Average Y					
			Is.	Me.	Ca.	Pro.	Dac.	Ch.
P. dayanus (males)								
0.85—0.88	4	0.8692	0.4352	0.5592	0.6603	0.4216	0.3472	1.1963
0.88—0.91	11	0.8971	0.4513	0.5776	0.6901	0.4512	0.3716	1.2223
0.91—0.94	11	0.9285	0.4624	0.5920	0.7060	0.4814	0.3947	1.2416
0.94—0.97	9	0.9574	0.4957	0.6343	0.7296	0.5153	0.4312	1.2732
0.97—1.00	9	0.9786	0.5338	0.6355	0.7507	0.5479	0.4655	1.2953
1.00—1.03	13	1.0111	0.5715	0.6928	0.7604	0.5795	0.4900	1.3277
1.03—1.06	1	1.0569	0.6201	0.7076	0.7931	0.6335	0.5185	1.3633
1.06—1.09	11	1.0738	0.6417	0.7335	0.8097	0.6450	0.5714	1.3871
1.09—1.12	5	1.1064	0.6862	0.7617	0.8286	0.6838	0.6060	1.4184
1.12—1.15	4	1.1414	0.7035	0.7955	0.8586	0.7380	0.6458	1.4537
1.15—1.18	4	1.1635	0.7376	0.8272	0.8856	0.7699	0.6832	1.4855
1.18—1.21	2	1.1902	0.7703	0.8500	0.9047	0.8021	0.7007	1.5093
1.21—1.24	2	1.2304	0.7952	0.8882	0.9364	0.8449	0.7698	1.5502
1.24—1.27	1	1.2577	0.7973	0.9031	0.9395	0.8513	0.7708	1.5559
P. dayanus (females)								
0.90—0.93	1	0.9031	0.4914	0.5185	0.7076	0.5911	0.3802	1.2504
0.93—0.96	1	0.9590	0.5315	0.5911	0.7451	0.6160	0.4314	1.2945
0.96—0.99	4	0.9765	0.5346	0.6041	0.7650	0.6308	0.4392	1.3064
0.99—1.02	7	1.0098	0.5727	0.6360	0.7899	0.6403	0.4707	1.3334
1.02—1.05	9	1.0378	0.6031	0.6622	0.8183	0.6574	0.4959	1.3564
1.05—1.08	14	1.0681	0.6311	0.6943	0.8267	0.6661	0.5314	1.3788
1.08—1.11	17	1.0936	0.6511	0.7233	0.8291	0.6736	0.5588	1.3960
1.11—1.14	9	1.1183	0.6756	0.7506	0.8561	0.6972	0.5680	1.4185
1.14—1.17	3	1.1572	0.7045	0.7707	0.8756	0.7193	0.6089	1.4437
1.17—1.20	1	1.1703	0.7243	0.7752	0.8865	0.7404	0.6434	1.4603
P. lamarrei (males)								
0.680—0.695	4	0.6880	0.1052	0.2670	0.4064	−0.0754	−0.0969	0.8633
0.695—0.710	4	0.7012	0.1380	0.2785	0.4425	−0.0630	−0.0862	0.8879
0.710—0.725	9	0.7234	0.1847	0.2923	0.4624	−0.0196	−0.0605	0.9177
0.725—0.740	1	0.7324	0.2040	0.3243	0.4770	0.0088	−0.0556	0.9344
0.740—0.755	11	0.7439	0.2232	0.3386	0.4997	0.0130	−0.0269	0.9553
0.755—0.770	13	0.7594	0.2587	0.3658	0.5069	0.0253	−0.0197	0.9691
0.770—0.785	10	0.7760	0.2878	0.3836	0.5477	0.0569	0.0043	0.9997
0.785—0.800	10	0.7895	0.3046	0.3962	0.5574	0.0676	0.0253	1.0162
0.800—0.815	6	0.8096	0.3584	0.4286	0.5966	0.0908	0.0607	1.0511
0.815—0.830	2	0.8228	0.3856	0.4579	0.6243	0.1206	0.0864	1.0828
0.830—0.845	2	0.8356	0.4058	0.4698	0.6384	0.1367	0.0969	1.0950
0.845—0.860	2	0.8451	0.4232	0.4911	0.6532	0.1461	0.1139	1.1122
P. lamarrei (females)								
0.800—0.820	4	0.8145	0.4133	0.4945	0.6627	0.1004	−0.0177	1.0994
0.820—0.840	3	0.8367	0.4393	0.5191	0.6931	0.1335	0.0212	1.1260
0.840—0.860	4	0.8558	0.4620	0.5391	0.7095	0.1673	0.0453	1.1468
0.860—0.880	8	0.8699	0.4728	0.5416	0.7193	0.1875	0.0682	1.1592
0.880—0.900	10	0.8926	0.4968	0.5563	0.7440	0.2253	0.0934	1.1828
0.900—0.920	8	0.9091	0.5185	0.5723	0.7576	0.2553	0.1106	1.1999
0.920—0.940	9	0.9333	0.5425	0.5883	0.7811	0.2906	0.1430	1.2240
0.940—0.960	14	0.9532	0.5630	0.6019	0.7951	0.3096	0.1703	1.2423
0.960—0.980	6	0.9692	0.5764	0.6232	0.8235	0.3284	0.1847	1.2615
0.980—1.000	4	0.9878	0.6016	0.6304	0.8338	0.3541	0.2201	1.2780
1.000—1.020	5	1.0120	0.6274	0.6503	0.8525	0.3874	0.2430	1.3016
1.020—1.040	5	1.0294	0.6468	0.6646	0.8774	0.4314	0.2833	1.3268

Table—1 (*Contd.*)

P. Kistnensis (males)

0.765—0.805	2	0.7888	0.3979	0.4116	0.5622	0.1492	0.0602	1.0512
0.805—0.845	4	0.8356	0.4302	0.4733	0.6045	0.2040	0.1052	1.0992
0.845—0.860	7	0.8547	0.4548	0.4872	0.6214	0.2304	0.1139	1.1190
0.860—0.875	15	0.8668	0.4579	0.4938	0.6393	0.2419	0.1418	1.1297
0.875—0.890	12	0.8784	0.4658	0.4978	0.6490	0.2552	0.1486	1.1387
0.890—0.905	4	0.8945	0.4927	0.5213	0.6532	0.2726	0.1614	1.1538
0.905—0.945	1	0.9085	0.5185	0.5315	0.6646	0.2788	0.1761	1.1673
0.945—0.985	3	0.9620	0.5539	0.5794	0.7236	0.3502	0.2377	1.2225
0.985—1.025	3	1.0198	0.6138	0.6432	0.7767	0.4216	0.2988	1.2801
1.025—1.065	2	1.0569	0.6314	0.6571	0.7986	0.4621	0.3304	1.3075

P. Kistnensis (females)

0.920—0.950	2	0.9368	0.5563	0.5854	0.7282	0.3520	0.1901	1.2201
0.950—0.965	2	0.9638	0.5680	0.6074	0.7404	0.3802	0.2304	1.2405
0.965—0.980	3	0.9743	0.5843	0.6170	0.7482	0.3979	0.2480	1.2526
0.980—0.995	5	0.9859	0.5933	0.6229	0.7505	0.4182	0.2647	1.2586
0.995—1.010	8	1.0010	0.5988	0.6294	0.7585	0.4292	0.2843	1.2683
1.010—1.025	7	1.0182	0.6170	0.6415	0.7627	0.4472	0.2993	1.2826
1.025—1.040	4	1.0273	0.6218	0.6603	0.7709	0.4655	0.3030	1.2909
1.040—1.055	10	1.0449	0.6351	0.6644	0.7837	0.4826	0.3483	1.3079
1.055—1.085	2	1.0682	0.6540	0.6946	0.7924	0.5184	0.3890	1.3310

The following values of equation (1) have been derived from the contents of Table 1 :

P. DAYANUS

Males :

Is. $y = 0.11471x^{(1.47633-0.03700x)} e^{0.09216}$

Me. $y = 0.80324x^{(0.75831+0.01122x)} e^{-0.02528x}$

Ca. $y = 0.58841x^{(0.97136-0.02199x)} e^{0.05596x}$

Pro. $y = 0.11331x^{(1.47314-0.03108x)} e^{0.08493x}$

Dac. $y = 0.14242x^{(1.31815-0.01511x)} e^{0.03836x}$

Ch. $y = 1.39701x^{(1.14042-0.01707x)} e^{0.04755x}$

Females :

Is. $y = 5.24951x^{(-0.12251-0.08638x)} e^{-0.20774x}$

Me. $y = 0.04292x^{(1.92579-0.09433x)} e^{0.23539x}$

Ca. $y = 0.80834x^{(0.86014-0.01898x)} e^{0.04636x}$

Pro. $y = 26.60003x^{(-0.65390-0.11689x)} e^{-0.30195x}$

Dac. $y = 0.01141x^{(2.64488-0.09781x)} e^{0.17522x}$

Ch. $y = 11.35090x^{(0.33670-0.04862x)} e^{-0.12822x}$

Table—1 (contd.)

P. LAMMARREI

Males :

Is. $y = 0.00602x^{(3.23471-0.21095x)} e^{0.37623x}$

Me. $y = 1.46407x^{(0.22711+0.19429x)} e^{-0.33327x}$

Ca. $y = 0.05556x^{(2.85567-0.13211x)} e^{0.22725x}$

Pro. $y = 0.06180x^{(1.62663-0.03558x)} e^{0.08315x}$

Dac. $y = 2.32794x^{(-0.51608+0.30904x)} e^{0.53837x}$

Ch. $y = 0.73892x^{(1.46082+0.01033x)} e^{-0.03505x}$

Females :

Is. $y = 0.39540x^{(1.01090+0.00728x)} e^{-0.01535x}$

Me. $y = 0.63626x^{(0.84624-0.00934x)} e^{0.01951x}$

Ca. $y = 1.22722x^{(0.74386+0.02716x)} e^{-0.05939x}$

Pro. $y = 0.19225x^{(1.08870+0.05248x)} e^{-0.11906x}$

Dac. $y = 0.27071x^{(0.78361+0.07113x)} e^{-0.15940x}$

Ch. $y = 3.16757x^{(0.78412+0.03106x)} e^{-0.07169x}$

P. KISTNENSIS

Males :

Is. $y = 0.15868x^{(1.44766-0.05605x)} e^{0.11551x}$

Me. $y = 0.05151x^{(2.02498-0.12318x)} e^{0.25637x}$

Ca. $y = 0.15778x^{(1.64443-0.08007x)} e^{0.16443x}$

Pro. $y = 0.14627x^{(1.23893-0.00778x)} e^{0.01001x}$

Dac. $y = 0.10764x^{(1.27621-0.03425x)} e^{0.04824x}$

Ch. $y = 0.88976x^{(1.33401-0.04403x)} e^{0.09577x}$

Females :

Is. $y = 0.42586x^{(0.97733-0.02046x)} e^{0.04610x}$

Me. $y = 56.49880x^{(-1.07954+0.19036x)} e^{-0.44871x}$

Ca. $y = 5.40513x^{(0.02768+0.04580x)} e^{-0.10632x}$

Pro. $y = 0.76230x^{(0.54748+0.06921x)} e^{-0.15996}$

Dac. $y = 0.50058x^{(0.60426+0.08725x)} e^{-0.20687x}$

Ch. $y = 18.17380x^{(-0.00219+0.07828x)} e^{-0.17783x}$

TABLE II

Showing the values of ρ for the cheliped and its segments

x	Is.	Me.	Ca.	Pro.	Dac.	Ch.
			P. dayanus (males)			
7.40	1.3365	0.8205	0.8970	1.3913	1.2664	1.1021
7.89	1.3085	0.8303	0.8809	1.3697	1.2553	1.0899
8.48	1.2732	0.8425	0.8607	1.3424	1.2413	1.0745
9.07	1.2368	0.8552	0.8398	1.3061	1.2268	1.0584
9.52	1.2077	0.8652	0.8229	1.2912	1.2152	1.0456
10.26	1.1584	0.8821	0.7946	1.2524	1.1956	1.0236
11.40	1.0786	0.9094	0.7485	1.1895	1.1639	0.9889
11.85	1.0458	0.9205	1.7295	1.1636	1.1508	0.9734
12.78	0.9766	0.9440	0.6894	1.1085	1.1232	0.9424
13.85	0.8934	0.9720	0.6413	1.0424	1.0900	0.9050
14.57	0.8357	0.9914	0.6078	0.9963	1.0670	0.8790
15.49	0.7601	1.0168	0.5640	0.9356	1.0367	0.8447
17.00	0.6319	1.0597	0.4896	0.8329	0.9855	0.7868
18.10	0.5351	1.0921	0.4334	0.7551	0.9467	0.7429
Mean	1.0199	0.9287	0.7142	1.1412	1.1403	0.9612
			P. dayanus (females)			
8.00	0.3437	1.4849	0.7636	−0.1896	−0.3208	0.5088
9.10	0.5090	1.3137	0.7280	0.0111	−0.0037	0.5894
9.47	0.5677	1.2529	0.7154	0.0828	0.1572	0.6182
10.23	0.6909	1.1248	0.6889	0.2336	0.4221	0.6790
10.91	0.8057	1.0052	0.6642	0.3749	0.6704	0.7359
11.70	0.9435	0.8616	0.6344	0.5448	0.9694	0.8045
12.41	1.0711	0.7283	0.6070	0.7028	1.2474	0.8683
13.13	1.2048	0.5883	0.5781	0.8686	1.5397	0.9354
14.36	1.4403	0.3417	0.5517	1.1618	2.0560	1.0541
14.80	1.5268	0.2511	0.5086	1.2695	2.2461	1.0979
Mean	0.9104	0.8953	0.6440	0.5060	0.8984	0.7892
			P. lamarrei (males)			
4.88	2.4103	1.0510	1.7985	1.4861	0.7541	1.5331
5.03	2.3527	1.1060	1.7612	1.4763	0.8405	1.5387
5.29	2.2501	1.2041	1.6947	1.4589	0.9941	1.5480
5.40	2.2060	1.2461	1.6662	1.4515	1.0601	1.5520
5.54	2.1484	1.3010	1.6014	1.4417	1.1463	1.5572
5.75	2.0639	1.3816	1.5743	1.4274	1.2727	1.5648
5.97	1.9710	1.4701	1.5143	1.4116	1.4118	1.5732
6.16	1.8904	1.5469	1.4622	1.3980	1.5323	1.5804
6.46	1.7640	1.6670	1.3807	1.3766	1.7210	1.5919
6.65	1.6758	1.7510	1.3237	1.3617	1.8528	1.5998
6.85	1.5863	1.8360	1.2660	1.3465	1.9864	1.6079
7.00	1.5180	1.9010	1.2220	1.3349	2.0886	1.6141
Mean	1.9864	1.4552	1.5221	1.4143	1.3884	1.5718

TABLE II—(Contd.)

P. lamarrei (females)

6.52	1.0473	0.7983	0.8658	1.2964	1.0780	0.9127
6.87	1.0518	0.7924	0.8819	1.3260	1.1187	0.9307
7.17	1.0559	0.7872	0.8965	1.3528	1.1558	0.9471
7.41	1.0591	0.7830	0.9081	1.3744	1.1854	0.9601
7.81	1.0647	0.7757	0.9281	1.4112	1.2361	0.9826
8.11	1.0690	0.7702	0.9436	1.4397	1.2754	1.0000
8.58	1.0758	0.7612	0.9681	1.4852	1.3379	1.0276
8.98	1.0819	0.7535	0.9897	1.5252	1.3930	1.0520
9.32	1.0870	0.7467	1.0084	1.5598	1.4405	1.0730
9.72	1.0934	0.7385	1.0311	1.6019	1.4983	1.0987
10.28	1.1023	0.7270	1.0632	1.6615	1.5803	1.1350
10.70	1.1091	0.7182	1.0879	1.7074	1.6434	1.1629
Mean	1.0748	0.7627	0.9644	1.4785	1.3286	1.0235

P. Kistnensis (males)

6.15	1.1872	1.4680	1.2687	1.2026	1.1528	1.1603
6.85	1.1161	1.3137	1.1669	1.1928	1.1209	1.1081
7.16	1.0835	1.2427	1.1200	1.1881	1.1063	1.0840
7.36	1.0619	1.1956	1.0890	1.1852	1.0966	1.0680
7.56	1.0401	1.1482	1.0577	1.1822	1.0867	1.0519
7.84	1.0087	1.0799	1.0127	1.1778	1.0727	1.0286
8.10	0.9795	1.0165	0.9708	1.1737	1.0616	1.0070
9.16	0.8550	0.7455	0.7923	1.1564	1.0039	0.9145
10.47	0.6920	0.3907	0.5588	1.1337	0.9312	0.7932
11.40	0.5704	0.1256	0.3845	1.1169	0.8769	0.7022
Mean	0.9594	0.9726	0.9421	1.1709	1.0510	0.9918

P. Kistnensis (females)

8.65	0.8173	0.2379	0.3588	1.0540	1.1977	0.5974
9.20	0.7954	0.4306	0.4061	1.1258	1.2853	0.6804
9.43	0.7862	0·5117	0.4260	1.1560	1.3222	0.7152
9.68	0.7759	0.6031	0.4484	1.1900	1.3638	0.7545
10.02	0.7616	0.7285	0.4791	1.2367	1.4209	0.8084
10.43	0.7444	0.8812	0.5166	1.2935	1.4904	0.8739
10.65	0.7348	0.9650	0.5370	1.3247	1.5285	0.9098
11.09	0.7157	1.1350	0.5787	1.3879	1.6059	0.9827
11.70	0.6884	1.3764	0.6377	1.4776	1.7158	1.0860
Mean	0.7577	0.7633	0.4876	1.2496	1.4367	0.8231

Table 3 gives the values of the equilibrium constant 'α' of the simple allometry equation for the second cheliped and its segments.

<center>TABLE III</center>

Showing the values of α for the cheliped and its segments

	Is.	Me.	Ca.	Pro.	Dac.	Ch.
			P. dayanus			
♂	1.0385	0.9231	0.7314	1.1631	1.1509	0.9725
♀	0.8906	0.9656	0.6520	0.5207	0.9490	0.7739
			P. la marrei			
♂	2.0052	1.4474	1.5394	1.4163	1.3799	1.5705
♀	1.0740	0.7638	0.9632	1.4770	1.3245	1.0262
			P. Kistnensis			
♂	0.9217	0.9152	0.8984	1.1654	1.0337	0.9644
♀	0.7586	0.7890	0.4895	1.2515	1.4366	0.8251

It may be noted that the values of the difference between α and the corresponding mean ρ, for the segments are very small and may be due to the fluctuations of sampling and the insufficient size of the data. The growth-gradient pattern obtained by taking centres of homologous regions at equal distances along the x-axis and the corresponding value of α along the y-axis, can therefore be said to be one obtained by properly pooling together a number of component patterns occurring for different values of x and y in the phase of growth.

It is seen that there is only one phase of growth in the male and the female chelipeds in each of the three species, other than P. hendersoni, with a progressive change in the successive values of the growth-coefficient. Text figures 1 and 2 have been drawn to show respectively the growth-gradient within the cheliped and the graph of $\dfrac{d\rho}{dx}$ against the carapace length x. These may be seen for the growth-gradient as follows :

A. *P. dayanus*

The phase of growth in the males starts with a growth-centre in the Pro. and a point of depression in the Me. which developes into a growth-centre towards the end. From the values of $\dfrac{d\rho}{dx}$ and variance (ρ), suitably estimated for the given sample size, it would be seen that in the males the Me. shows a progressive increase in its relative rate of growth and ultimately is the only positively heterogonic joint. Other joints lose in the values of their relative growth rates, the loss being most marked in the Is. which is a high point at the start of the phase. Thus in the basal region of the organ, consisting of the Is. and the Me., this helps in checking the Is. from attaining a great length which may be quite out of proportion with that of the Me. In the distal region forming the chela (i.e. the Pro. and the Dac.) the Pro. shows a more marked decrease in the value of the relative growth rate than the Dac. This similarly checks the Pro. from dominating over the Dac. which starts with a lower growth rate as well as the length.

The gradient in the female organ is much different from that in the male in that it starts with a growth-centre in the Me. and a point of depression in the Dac. which later developes into a steep high point forming a region of greatest activity. The relative growth rates for the Is. and the Pro. also show progressive increase in their values, it being more marked in the latter joint. In the basal region the Is. forms a crusher joint.

The order of the values of ρ changes from (Me., Ca., Dac., Is., Pro.) at the start of the phase to (Ca., Is., Pro., Dac., Me.) at the end of it, in the male segments; while in the female it changes from (Dac., Pro., Is., Ca., Me.) to (Me., Ca., Pro., Is., Dac.). The higher value in the female cheliped than in the male of a suitably calculated variation in the component growth-gradient patterns and the comparison of the values of the rank correlation between the initial and the final orders of ρ in the two chelipeds show that the gradient in the female is much more marked than the one in the male. This shows a much mor stable state of growth in the male organ than in the female one, and is associated with a more rapid shift of the growth-centre from the basal region to the distal region in the female cheliped than the one from the distal to the basal region in the male cheliped.

B. *P. lamarrei*

The growth-gradient in the male cheliped starts with a growth-centre in the Ca. and, as in the males of P. dayanus, a point of depression in the Me. which develops into a growth-centre towards the end of the phase. There is a progressive increase in the value of the relative growth rate for the Me. and the Dac. it being greater in the latter joint which develops into a high point at the end of the phase. The Is. forms a high point at the start but loses in its relative growth rate value most rapidly. In the basal region the Me. shows a progressively increasing value of the relative growth rate and this helps in controlling the Is. from becoming the crusher over the Me. (compare with P. dayanus). In the distal region the progressively increasing ρ coupled with its high variance and rapidly increasing $\dfrac{d\rho}{dx}$ makes the Dac. a distinct crusher joint.

There is no change in the growth-gradient pattern throughout the phase, thus showing a stable state of growth in the female cheliped of this species. Pro. forms a growth-centre here. The positively heterogonic joints here are the Is., Dac. and the Pro., and later the Ca. also developes into the same. These joints show progressive increase in the values of their relative growth rates, it being most marked in the Dac., in the basal region the Is. forms the crusher.

It is seen that the order of the values ot ρ changes from (Dac., Me., Pro., Ca., Is.) to (Ca., Pro., Is., Me., Dac.) in the male cheliped, whereas in the female it remains the same throughout the phase, viz., (Me., Ca., Is., Dac., Pro.). This shows that the gradient in the female cheliped remains perfectly unchanged for all lengths of the cheliped whereas in the male it is a changing one and is associated with a rapid shift of the growth-centre from the central (Ca.) to the basal (Me.) region.

C. *P. Kistnensis*

In the male cheliped it starts with a growth-centre in the Me. which becomes a point of depression between the neighbouring joints the Is. and the Ca. towards the end of the phase when the Pro. forms a growth-centre and remains as the only positively heterogonic joint. There is a progressive decrease in the values of the relative growth rate for all the joints.

In the female organ the Me. which is a point of depression at the start, developes into a growth-centre towards the end of the phase when the positively heterogonic

joints are the Me., Ca., Pro. and the Dac., of which the last forms a high point throughout the phase. Except the Is. all the joints show progressive increase in the values of their relative growth rates, it being greatest in the Me. In the basal region, as in the males of P. dayanus and P. lamarrei, the Is. is kept from attaining a conspicuously larger length than the Me.

The order of the values of ρ changes from (Dac., Is., Pro., Ca., Me.) to (Me., Ca., Is., Dac., Pro.) in the male cheliped and from (Me., Ca., Is., Pro., Dac.,) to (Ca., Is., Me., Pro., Dac.). The correlation between the initial and the final orders is negative in the male and positive in the female. Also, the variation in the growth-gradient patterns in the male cheliped is much higher than one in the female. These show that the gradient in the male cheliped is highly marked (as is associated with a rapid shift of the growth-centre from the Me. of the basal region to the Pro. of the distal region), whereas in the female cheliped the gradient is very stable and is associated with only a slow development of heterogony in the Me. and a progressive and gradual change in the value of the relative growth rate for each of the other segments of the organ.

It has already been mentioned by the author (op. cit.) that in P. hendersoni the gradient in the male cheliped (I Phase) is more marked than in the females In fact, in the male cheliped (I Phase) there takes place a rapid progress in the value of the relative growth rates in the segments of the distal region, a steep fall from a positive to a negative heterogony in the central region and a development of the growth-centre in the Me. of the basal region. In the second phase of growth of the male cheliped, however, a very high value of the variation in the component growth-gradient patterns and a positive rank correlation between the initial and the final orders of the values of ρ show a highly marked growth-gradient progressing in one direction only and resulting in the progress from a less to a more steep growth-centre at the Pro. of the distal region and a development of another, but less marked, growth-centre at the Me. of the basal region of the organ.

REFERENCES

Champy, C. (1922). Thyroid. *Arch. Morph. Gen. Exp.* 1922.
———— (1924). Sexualite at Hormones. Paris : G. Doin.
Hexley, Julian S. (1932). Problems of Relative Growth, Methuen & Co. Ltd., London.
Misra, R. K. (1957). An expression for the growth-coefficient 'a' in the law $y = bx^a$ of constant differential growth ratio, expressing the growth relationship between the body size x and the organ size y, in various organic forms. *Proc. Nat. Inst. Sci. India*, **23B**, 42–47.
———— (1958). A new approach to the study of the growth-gradient in the segments of the second pair of chelipeds of the Indian freshwater prawns, *Palaemon* hendersoni DeMan (Crustacea : Decapoda Palaemonidae). *Ibid.*, **24 B**, 67-78.
Prizibram, H. (1902). Intraindividuelle Variabilitat der Carapaxdimensionen bei brachyuren Crustaceen. *Arch. EntwMech.*, **13**, 588.
———— (1917). Wachstumsdessungen an Sphodromantis bioculata Burm. III. Lange regenerierender und normaler Schreitbeine. *Ibid.*, **43**, 1.
Tazelaar, M. A. (1930). The relative growth of parts of *Palaemon* Carcinus. *Brit. Expmtl. Biol.*, **7**, 165.
Thompson, D'Arcy W. (1917, 1942). Growth and Form. Cambridge Univ. Press.
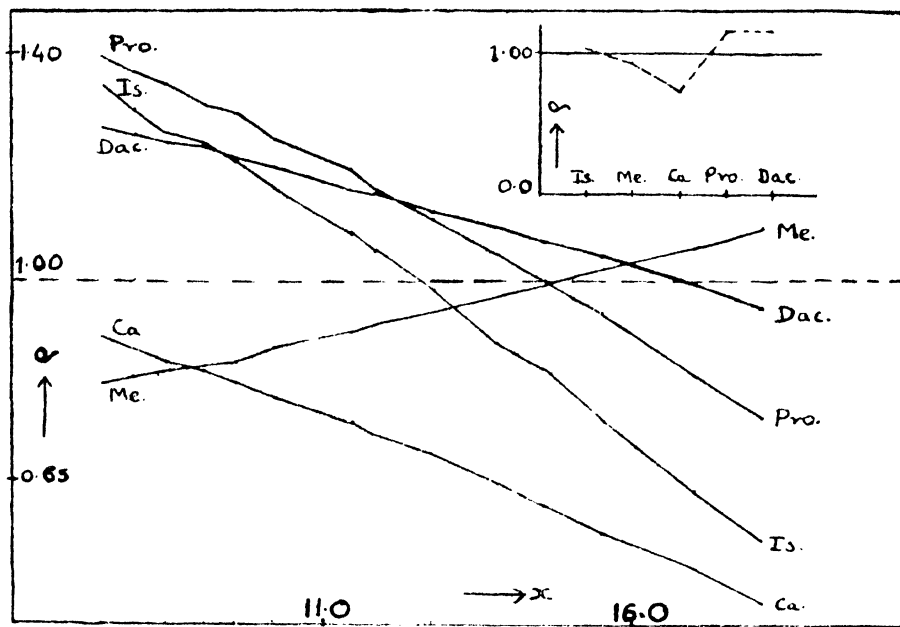
TEXT-FIG. 1(a).

Growth-gradient within the cheliped in
P. dayanus henderson (male).
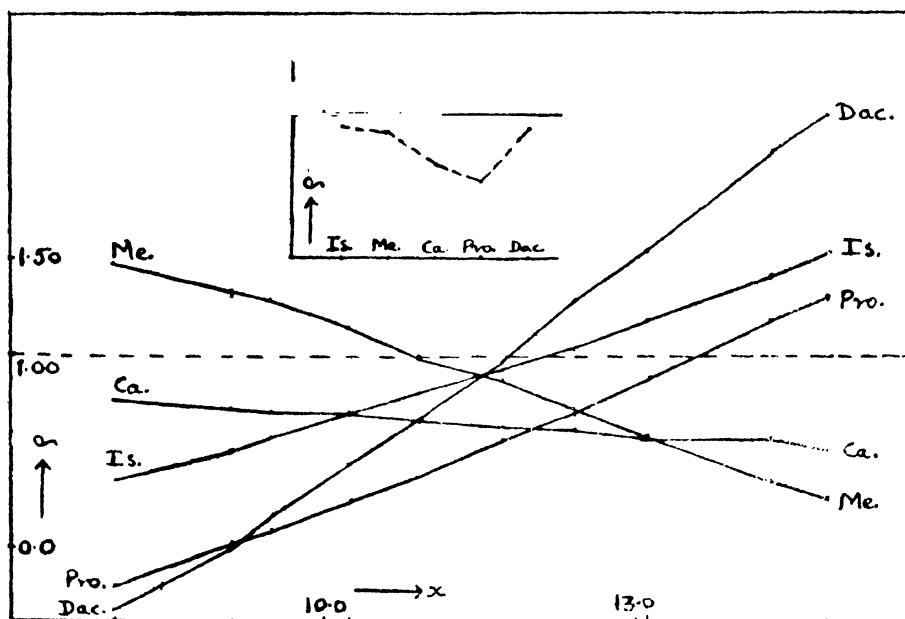
TEXT-FIG. 1(b).

Growth-gradient within the cheliped in
P. dayanus henderson (female).
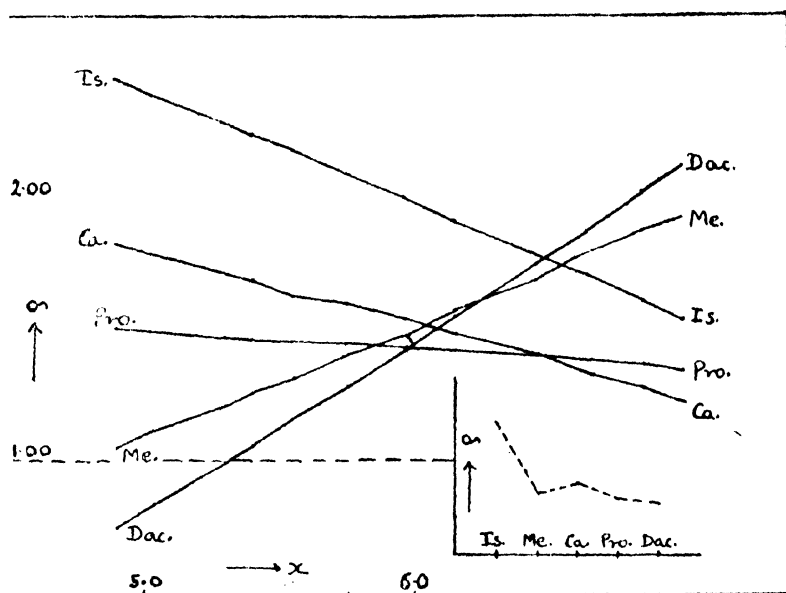
TEXT-FIG. 1(c).

Growth-gradient within the cheliped in
P. lamarrei H. Milne-Edwards (male).
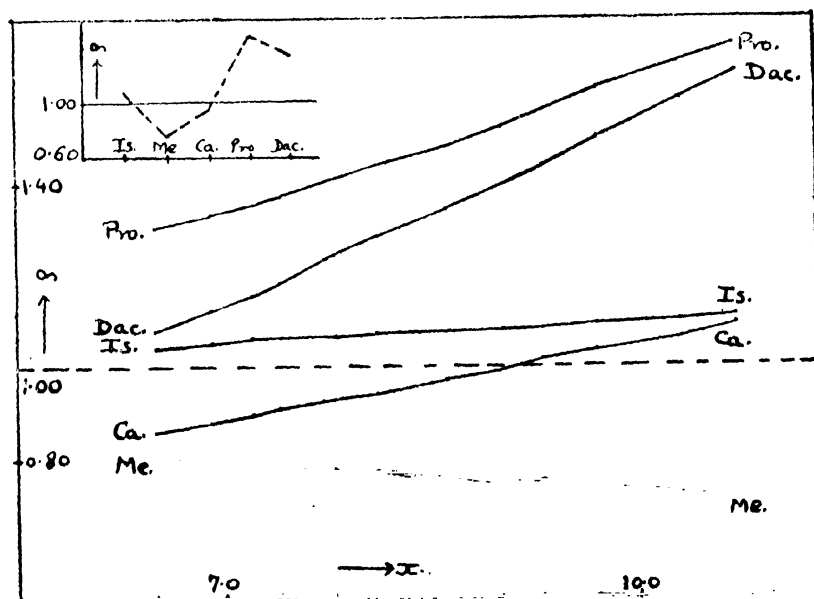
TEXT-FIG. 1(d).

Growth-gradient within the cheliped in
P. lamarrei H. Milne-Edwards (female).
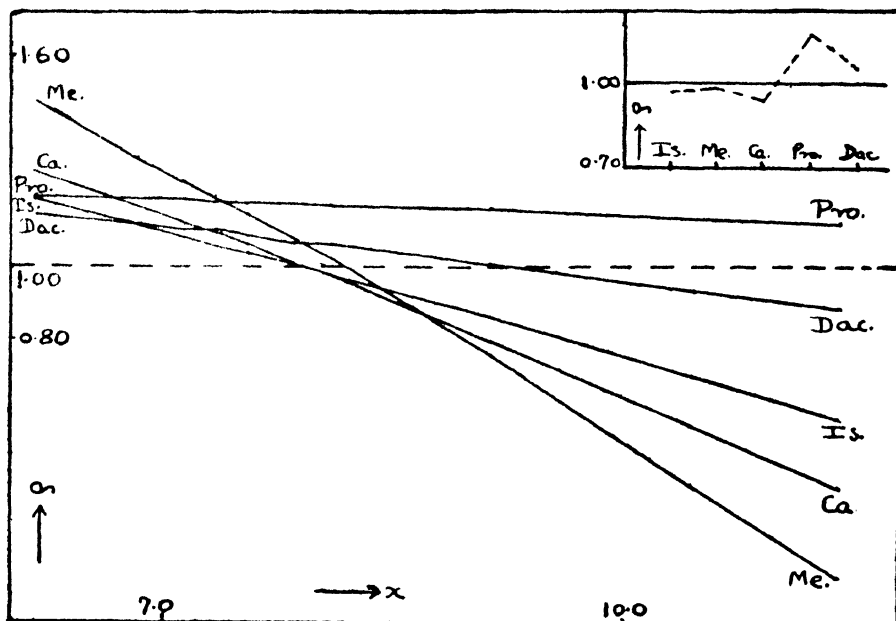
TEXT-FIG. 1(e).

Growth-gradient within the cheliped in
P. Kistnensis Tiwari (male).
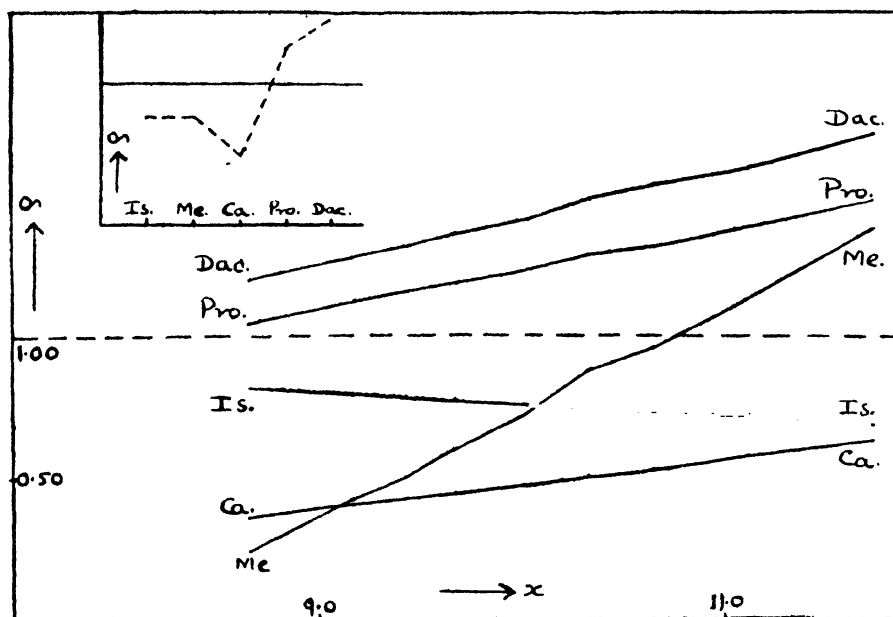
TEXT-FIG. 1(f).

Growth-gradient within the cheliped in
P. Kistnensis Tiwari (female).
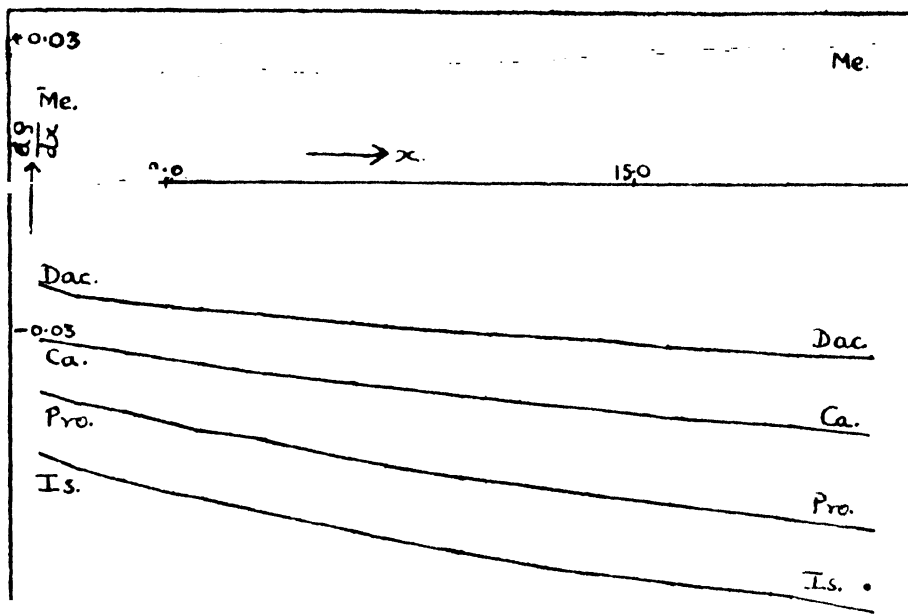
TEXT-FIG. 2(a).

Graph of $\frac{d\rho}{dx}$ against the carapace length x in

P. dayanus henderson (male).
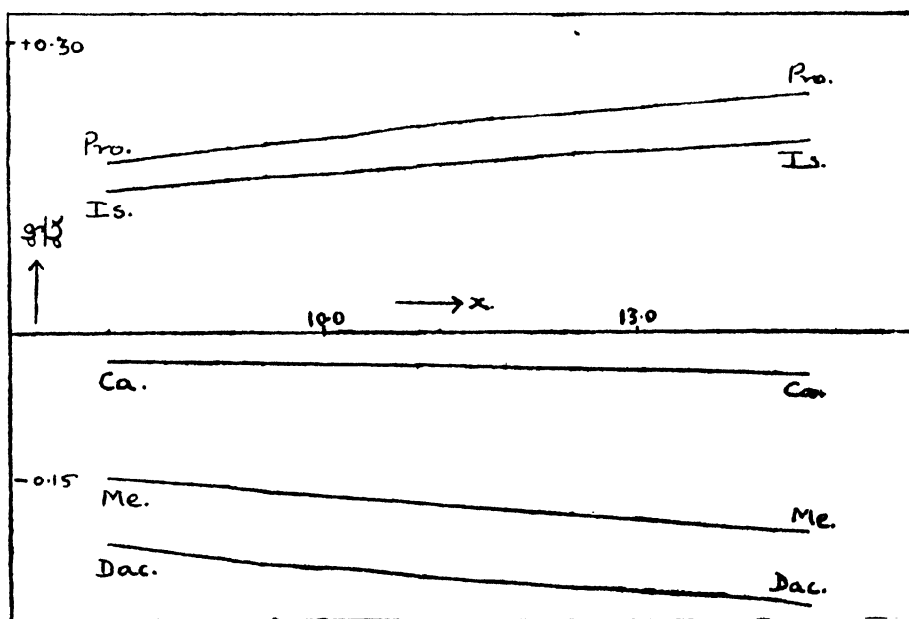
TEXT-FIG. 2(b).

Group of $\frac{d\rho}{dx}$ against the carapace length x in

P. dayanus henderson (female),
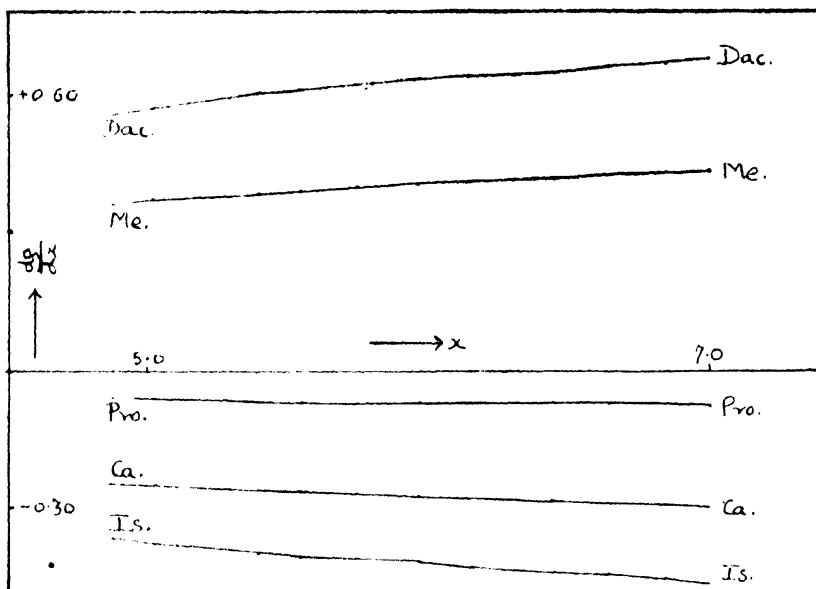
TEXT-FIG. 2(c).

Graph of $\dfrac{d\rho}{dx}$ against the carapace length x in

P. lammarrei **H**. Milne-Edwards (male).
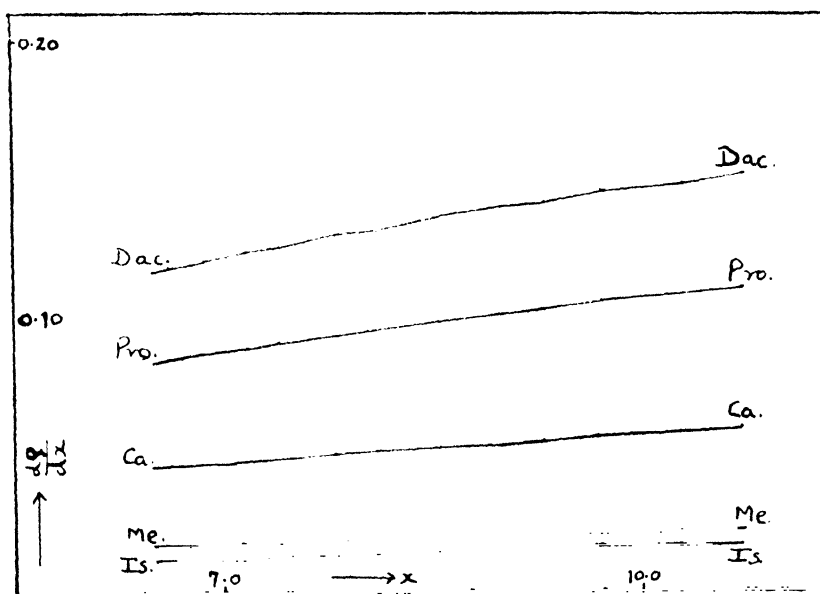
TEXT-FIG. 2(d).

Graph of $\dfrac{d\rho}{dx}$ against the carapace length x in

P. lamarrie **H**. Milne-Edwards (female).
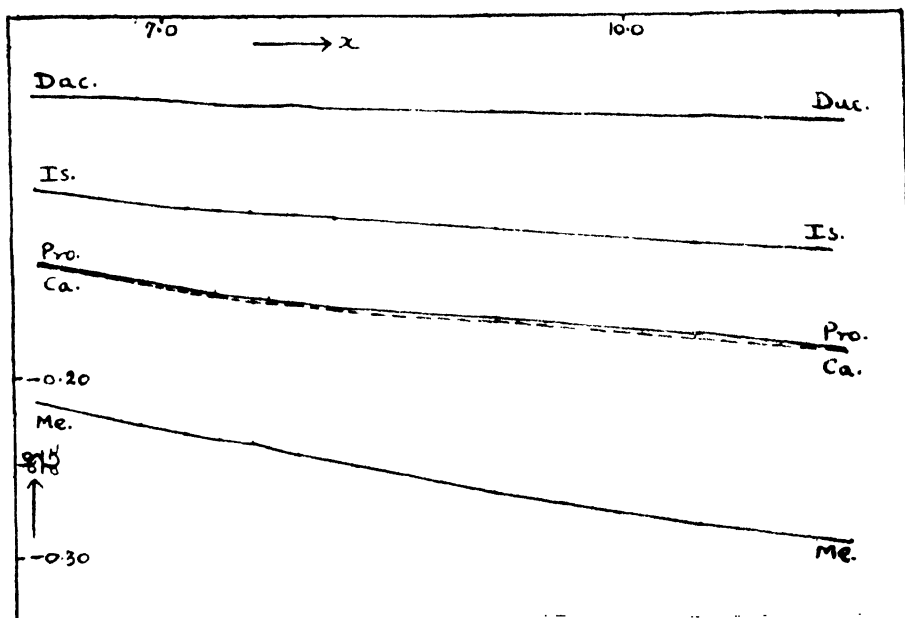
TEXT-FIG. 2(e).

Graph of $\dfrac{d\rho}{dx}$ against the carapace lenth x in

P. Kistnensis Tiwari (male).

TEXT-FIG. 2(f).

Graph of $\dfrac{d\rho}{dx}$ against the carapace length x in

P. Kistnensis Tiwari (female).

ON THE SWIMBLADDER AND ITS CONNECTION WITH THE INTERNAL EAR IN FAMILY CICHLIDAE

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(Communicated by M. L. Bhatia, F. N. I.)

ABSTRACT

The swimbladder of the genus *Etroplus* of the family Cichlidae is described in detail. Work has also been carried out on *T. mossambica*, an African member of Cichlidae.

The generic variations in the swimbladder may be of systematic value. The specific variations in the structure of the swimbladder within the genus *Etroplus* have also been emphasized.

The two chambers of the swimbladder, partitioned by a perforated diaphragm, appear to be functionally different. The large and thicker anterior chamber lodges the gas gland and retia mirabilia and serves for the secretion of the gases. The posterior chamber, smaller and thinner, is the seat of gas resorption.

Microscopical study of the swimbladder reveals the usual histological structures.

The peculiar nature of the ear-swimbladder relation by means of auditory cacca which lie apposed to the tendinous pads, in the genus *Etroplus*, has been described for the first time, which is characteristic of its own.

The dual function of the tendinous pads which plug the auditory fontanelle is explained.

It has been possible to amplify the description of the swimbladder of the genus *Etroplus* given by Day (1889).

INTRODUCTION

The present paper is second of the series, in which a general morphological and histological study of the swimbladder of the Indian teleostean fishes has been undertaken. The first record of this series is on the swimbladder of genus *Notopterus* (Lacepede) (Dehadrai, 1957).

In the course of investigations on the swimbladder of common teleostean fishes of India, certain peculiarities were noticed in the structure of the swimbladder of Cichlid fishes. Considering the paucity of literature on the structure of the swimbladder of Cichlid fishes, the present work on the genus *Etroplus** (Cuv. & Val.), was attempted. Investigations were done also on *Tilapia mossambica* (Andrew Smith), an African member of Cichlidae.

HISTORICAL RESUME

Authors like Gunther (1861), Regan (1905a, b, c ; 1906d, e ; 1913d), Bridge and Boulenger (1910), Weber and de Beaufort (1922), Trewavas (1933), Berg (1940) etc., have made no reference to the structure of the swimbladder in their account of the family Cichlidae. Day (1889) described the swimbladder of genus *Etroplus* as "the air vessel present, large and simple".

MATERIAL AND METHODS

Only one genus of Cichlid fishes, namely, *Etroplus* is found in India. It includes only three species, *E. suratensis* (Bloch), *E. maculatus* (Bloch), *E. canarensis* (Day), all of which are confined to South India. A recently introduced African member of Cichlidae *Tilapia mossambica* is being cultured in a few states of India like Bengal and Kerala.

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* Formerly, genus *Etroplus* was included in family Chromidae.

Specimens preserved in formalin were obtained from Kerala. A few specimens fixed in Bouins' picro-aceto-formol solution, were also procured from Kerala for histological studies.

For morphological studies, the fishes were dissected laterally so as to expose the swimbladder. For histological studies, sections were cut at 6μ—8μ and were stained in Delafield's haematoxylin and counterstained by alcoholic eosin. Borax carmine was used for whole flat mounts.

Gross Anatomy

Externally, the swimbladder of genus *Etroplus* is a large, smooth sac with a satiny texture, lying deep in the abdominal cavity (Fig. 1). A specimen of *E. suratensis* measuring 16.3 cm. has a swimbladder 5.7 cm. long. In genus *Etroplus*, the swimbladder forks anteriorly into two tubular caeca, which extend beyond the abdominal cavity and establish an anatomical relation with the auditory capsules (Fig. 1). Posteriorly, the swimbladder arches gently and gets firmly attached with the first haemal arch.
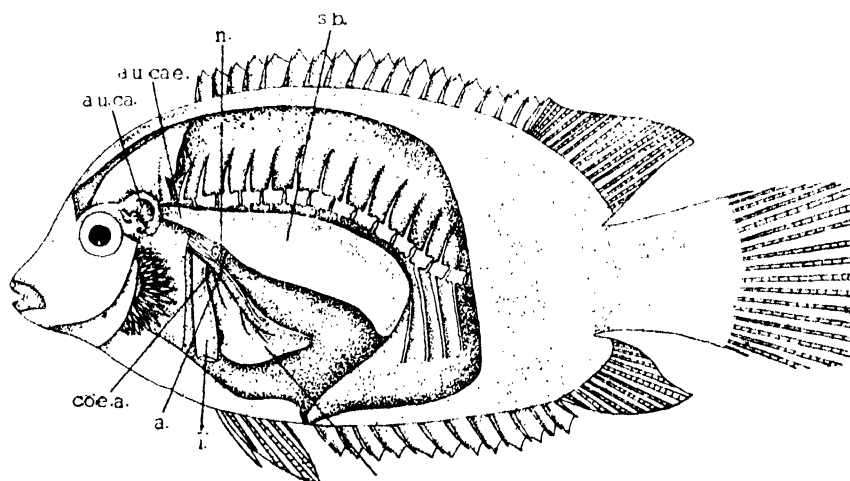
TEXT-FIG. 1.

Diagram showing the swimbladder and its associated organs in *Etroplus suratensis*. *a.c.*, alimentary canal; *a.*, artery; *au.ca.*, auditory capsule; *au.cae.*, auditory caecum, *coe.a.*, coeliaco-mesenteric artery; *n.*, nerve; *sb.*, swimbladder.

The swimbladder of *T. mossambica* is similar to that of genus *Etroplus* in structural details, except for a few differences. In a specimen of *T. mossambica* measuring 17 cm. the swimbladder is 5.8 cm. long. Moreover, the anterior forked caeca in *T. mossambica* do not reach upto the auditory capsules and as such the ear-swimbladder relation in this fish is wanting (Fig. 2).

In the genus *Etroplus*, on the mid-ventral line of the swimbladder runs a cord of tendon, which ascending along the posterior curve of the swimbladder is continuous with a similar cord on the mid-dorsal line. This dorsal tendinous cord bifurcates in front. Each of the branches courses along the inner margin of the

auditory caecum of its side and enters through the outer opening in the auditory
fontanelle in the form of an oval, flattened tendinous pad (Fig. 3b). . The auditory
fontanelle is a large cavity, situated on the posterior wall of the auditory capsule
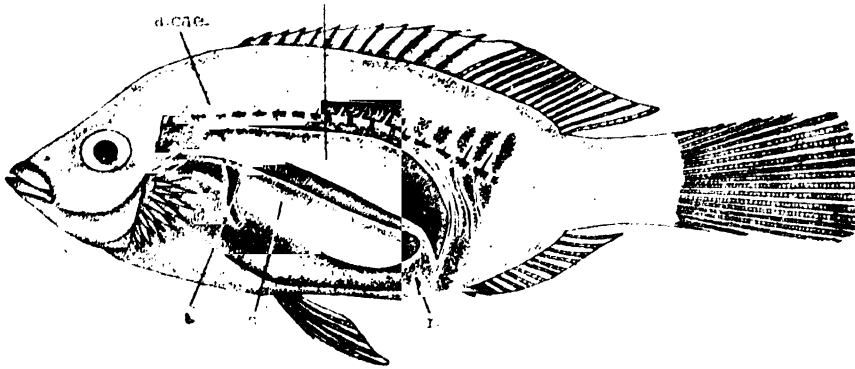
TEXT-FIG. 2.

Diagram showing the swimbladder and its associated organs in *Tilapia mossambica*
a.c., alimentary canal; *a.cae.*, anterior caecum; *sb.*, swimbladder.
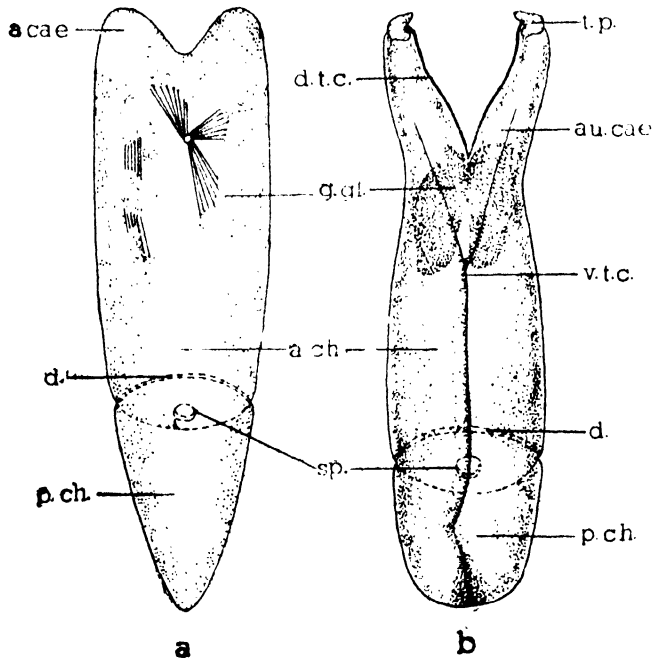
TEXT-FIG 3.

a—Swimbladder of *Tilapia mossambica* (ventral view). *a.ch.*, anterior chamber; *a.cae.*,
     anterior caecum; *d*, diaphragm; *g.gl.*, gas gland; *p.ch.*, posterior chamber; *r.*, *retia*;
     sp., sphincter.
b.—Swimbladder of *Etroplus suratensis a.ch.*, anterior chamber; *au.cae.*, auditory caecum;
     d., diaphragm; *d.t.c.*, dorsal tendinous cord; *g.gl.*, gas gland; *p.ch.*, posterior chamber;
     sp., sphincter; *t.p.*, tendinous pad; *v.t.c.*, ventral tendinous cord;

on either side of the occipital condyle. The tendinous pad fits as a plug, its rim fitting snugly into a shelf situated deep in the auditory fontanelle and thus closing the communication between it and the auditory capsule.

Internally, the swimbladder in the family Cichlidae, consists of an anterior and a posterior chamber separated by a well developed perforated diaphragm. The posterior chamber is about one fourth of the total length of the swimbladder. The anterior chamber communicates with the posterior chamber through a perforation which is a sphincter, situated more towards the ventral side of the diaphragm (Fig. 3a, b).

The sphincter in the genus *Etroplus* is formed by six or seven circular bands of muscle fibres and a few scattered tangentially radiating muscle fibres (Fig. 4b). But the sphincter in the swimbladder of *T. mossambica* is very strong and prominent. It is made of a number of concentric rings of thick muscle fibres and about seventeen thick, radiating bands of muscle fibres (Fig. 4a). These two sets of muscle fibres are interwoven and some of the radiating muscle bands ramify at the periphery.
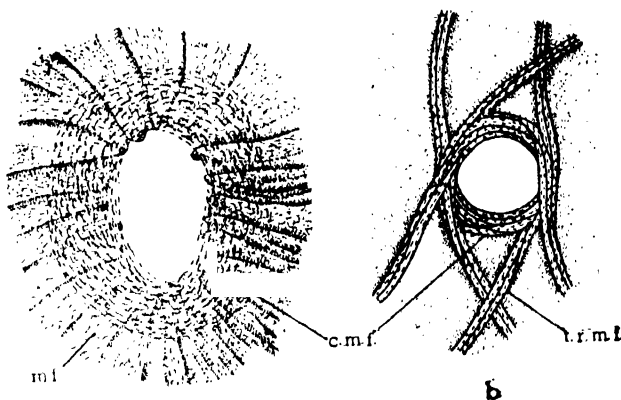
TEXT-FIG. 4.

a.—Diagram of the whole mount of the diaphragm of *T. mossambica* showing the sphincter. *c.m.f.*, circular muscle fibres; *r.m.f.*, radiating muscle fibres.
b.—Diagram of the whole mount of the diaphragm of *E. suratensis* showing the sphincter. *c.m.f.*, circular muscle fibres; *t.r.m.f.*, tangentially radiating muscle fibres.

The anterior chamber lodges the gas gland and the retia mirabilia. In *E. suratensis* (Fig. 3b), the gas gland is horse-shoe shaped and is disposed on the ventral wall of the swimbladder, while in *E. maculatus*, the gas gland is arranged in two rows of isolated patches. But in *T. mossambica*, the gas gland is disposed on the ventral wall of the swimbladder in a number of scattered patches of irregular shape and size (Fig. 3a).

Arterial supply to the swimbladder is from the coeliaco-mesenteric artery. The blood vessel, the pneumatic artery, enters the swimbladder at its anterior end, a little behind the forked caeca and breaks up into myriads of capillaries which form the retia mirabilia (Fig. 1).

The venous blood is drained by a vessel from the posterior chamber. The pneumatic vein thus formed eventually joins the posterior cardinal vein.

The swimbladder is innervated by the intestinal rami of the vagus nerve of each side. The nerve enters the swimbladder along with the arterial blood vessel to the organ (Fig. 1).

HISTOLOGY

Swimbladder wall :

The wall of the swimbladder is thick and consists of two distinct layers, the tunica externa and tunica interna. The tunica externa, a thick tough coat, is formed of elastic fibres and is highly coated with guanine. The delicately thin tunica interna consists as usual of,

a. an outer, loose, jelly-like layer of fibro-elastic tissue,
b. a narrow, circular layer of muscle fibres, the muscularis mucosa,
c. epithelial layer.

The tunica interna of the posterior chamber is comparatively thinner than that of the anterior chamber and is abundantly supplied with blood capillaries.

Gas gland and Retia mirabilia :

The gas gland (Fig. 5) consists of a mass of large polygonal cells forming a many layered glandular structure, situated near the muscularis mucosa. The gas gland is distributed on the ventral wall of the swimbladder in patches, which are of definite shape and size for different species. Each gas-gland-cell has a prominent nucleus.
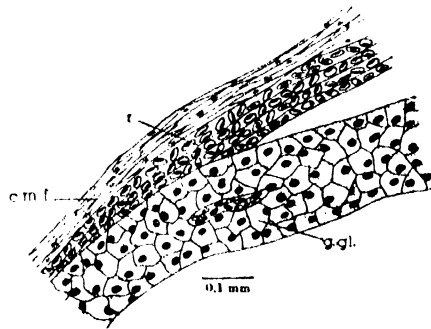
TEXT-FIG. 5.

Transverse section of the tunica interna of the swimbladder of *E. suratensis* through the retia and gas gland region. *c.m.f.,* circular muscle fibres; *r.,* retia; *g.gl.,* gas gland.

The patches of the gas gland draw their blood supply from the retia mirabilia, which are formed by the large number of blood capillaries.

CONNECTION OF THE SWIMBLADDER WITH THE INTERNAL EAR

Membranous labyrinth of ear in genus *Etroplus* consists, as usual, of the utriculus, the semicircular canals, and the sacculus. It is lodged in the auditory capsule formed by the otic bones. On the posterior wall of the auditory capsules are the auditory fontanelle, which open near the utriculus.

Each of the auditory caeca proceeds forward to establish a connection with the auditory capsule of its side. Tunica externa of each of the auditory caeca is very tough and ceases abruptly by becoming inserted into the outer rim of the auditory fontanelle. But the tendinous cord of the tunica externa of each of the auditory caeca enters the auditory fontanelle of its side from near the inner margin and proceeds forward and dilates to form the tendinous pad, which closes the opening of the auditory capsules described above. The presence of this pad closing the inner opening of the auditory capsule, converts the cavity of the auditory

fontanelle into a cup, which lodges the blind end of the tunica interna. Thus the ear-swimbladder relation in the genus *Etroplus* (Fig. 6), is through a close apposition of the blind end of the auditory caeca to the outer side of the tendinous pad in the
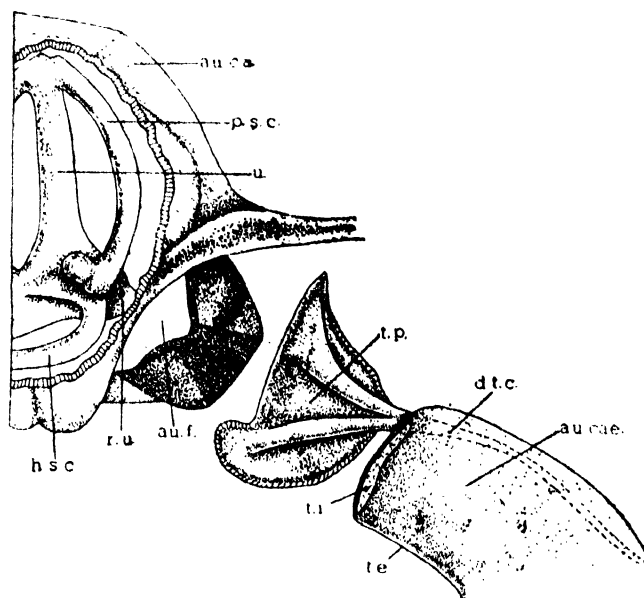
TEXT-FIG. 6

Diagram showing the ear-swimbladder relationship in *E. suratensis* with the auditory caecum separated from the auditory fontanelle. *au.ca.*, auditory capsule; *au.cac.* auditory caecum; *au.f.*, auditory fontanelle; *d.t.c.*, dorsal tendinous cord; *h.s.c.*, horizontal semicircular canal; *p.s.c.*, posterior semicircular canal; *r.u.*, recessus utriculi; *t.e.*, tunica externa; *t.i.*, tunica interna; *t.p.*, tendinous pad; *u.* utriculi.

respective auditory fontanelle, while the inner surface of this pad is bathed by the perilymph surrounding the membranous labyrinth of the ear.

DISCUSSION

The teleostean swimbladder has been a considerably debated organ owing to its diversity of form and functions. The swimbladder of Cichlid fishes presents an interesting and more complicated structure than that described by Day (1889). By the present work it has been possible to elucidate the complex structural disposition of the organ in Indian Cichlids.

The Cichlid fishes of the genus *Etroplus*, which constitute one of the excellent food fishes of India, have laterally compressed body. The well developed, large swimbladder provides them with an efficient adjustable float. The recently introduced African member of Cichlidae, *T. mossambica*, offers a fascinating comparative study of the swimbladder of Cichlid fishes available in India. The general structural disposition of the swimbladder in two genera *Etroplus* and *Tilapia* is remarkably similar. But the absence of the ear-swimbladder relation in *T. mossambica*, may be mentioned as a definite generic difference in the structure of the swimbladder. Further, the specific differences within the genus *Etroplus*, is evidenced by the variety in the disposition of the gas gland and retia mirabilia patches in the swimbladder of different species. But the scarcity of Cichlid fauna in the Indian waters'

hinders a more detailed study which may throw light on the systematics of this group.

The presence of a tough tendinous cord on the dorsal and ventral sides of the swimbladder in genus *Etroplus*, is very characteristic of its own. It appears to give considerable support to the swimbladder in maintaining its form. Moreover, the auditory caeca of the swimbladder are also supported by the bifurcation of the dorsal tendinous cord. The auditory caeca are firmly attached to the skull by means of the tendinous pads, which also plug the opening of the auditory capsules.

The two chambers of the swimbladder partitioned by a well developed diaphragm which possesses a sphincter, suggest the complex modification of the swimbladder in Cichlidae. The large anterior chamber, which possesses the well-developed retia mirabilia and the gas gland, is prominently modified as a gas secreting chamber. The intimate association between the gas gland tissue and the smooth muscle layer (muscularis mucosa) in the swimbladder of Cichlid fishes is worth mentioning. Its probable importance in the gas secretion (Fange, 1958), is that the movements of muscularis mucosa could have a mechanical effect upon the release of newly formed gas bubbles into the bladder. The posterior, thin walled, small chamber, which has a copious supply of the blood capillaries, provides a surface for the resorption of gas.

The passage of gas from one chamber to the other is controlled by the contraction and relaxation of the differently arranged muscle fibers of the sphincter that surround the perforation of the diaphragm. The comparative study of the structure of the sphincter in genus *Etroplus* and genus *Tilapia* clearly shows the definite functional efficiency of the sphincter mechanism in the swimbladder of *T. mossambica*. Generally the perforation of the diaphragm in teleostean fishes occupies a central position. But in the specimens of Cichlids examined, the aperture was found to be present more towards the ventral periphery of the diaphragm.

The most significant part of the organisation of the organ in genus *Etroplus* is its relation with the internal ear. The simple mode of coupling between the swimbladder and the ear by means of two anterior diverticula of the bladder wall which are directly applied to the auditory capsules, has been reported in many teleostean fishes like *Megalops* (de Beaufort, 1909), Notopteridae (Bridge, 1900 ; Dehadrai, 1957), the subfamily Morinae of Gadidae, (Parker, 1882 ; Hagman, 1921; Svetovidove, 1937), the Berycidae and Priacanthidae, (Stannius, 1854), and the Sparidae, (Weber, 1820). etc. In addition to the examples mentioned above, genus *Etroplus* of the family Cichlidae may also be included in the above category.

But a detailed study of the connection of the swimbladder of genus *Etroplus* with its internal ear has brought to light certain modifications, which are characteristics of its own. And thus this mode of ear-swimbladder relation in the genus *Etroplus* stands out quite different from those described by the earlier workers. The auditory caeca are not simply inserted into the auditory fontanelle, but are held firmly by the tendinous pads which fit into the auditory fontanelle. Moreover, in place of the usual fibrous tissue membrane covering the auditory fontanelle, these tendinous pads serve as plugs closing the openings of the auditory capsules. Thus the dual function of the tendinous pad is remarkable.

A number of dissections have confirmed that in genus *Etroplus*, the auditory fontanelle communicates with the auditory capsules, internally near the utriculus, which suggests the obvious relation of the swimbladder with the pars superior. And a refinement of hydrostatic perception may be attributed to this elaborate mechanism of ear-swimbladder relation in genus *Etroplus*. On the basis of the above mentioned anatomical evidences, it may be suggested that fishes of the genus *Etroplus* may have certain advantages in pressure perception and manipulation of air-volume in the swimbladder over *T. mossambica*, in which the ear-swimbladder relation is lacking.

ACKNOWLEDGEMENTS

REFERENCES

Berg, L. S. (1940). Classification of Fishes both Recent and Fossil, pp. 476, Moscow.

Bridge, T. W. and Boulenger, G. A. (1910). Fishes. Cambridge Natural History, Vol. VII, pp. 139–727.

Bridge, T. W. (1900). The air-baldder and its connection with the auditory organ in *Notopterus borneensis*. *J. Linn. Soc. (Zool.)*, **27**, 503.

Day, Francis, (1889). Fauna of British India. Fishes, II, 428–431.

Dehadrai, P. V. (1957). On the Swimbladder and its relation with the Internal Ear in genus *Notopterus* (Lacepede) *J. zool. Soc. India*, **9** (1) 50–61.

De Beaufort, L. F. (1909). Die Schwimmblase der Malacopterygii. *Morph. Jb.* **39**, 526

Gunther, A. (1861). Catalogue of the fishes in the Birtish Museum, IV, 264–266, London.

*Hagman, N. (1921), Studien uber die Schwimblase einiger Gadiden and Macruriden. *Akad. Abhand. Lund*, 1–124.

Jones, F. R. H. and Marshal, N. B. (1953). Structure and functions of the Teleostean Swim-bladder. *Biol. Rev.*, **28**, 16–83.

*Parker, T. J. (1882). On the connection of the air bladder and the auditory organ in the red cod (*Lotella bacchus*). *Trans. N. Z. Inst.*, **15**, 234.

Regan, C. T. (1905a). A revision of the fishes of the South American Cichlid genera *Acara, Nanacara, Acoropsis*, and *Astronotus*. *Ann. Mag. nat. Hist.*, (7), **15**, 329–427.

———— (1905b). A revision of the fishes of the South American Cichlid genus *Cichlosoma* and of the allied genera. *Ibid.*, (7), **16**, 60–77, 225–243, 316–340.

———— (1905c). A revision of the fishes of the South American Cichlid genera, *Crenacara, Batrachops* and *Creniciahla*. *Proc. zool. Soc. Lond.*, pp. 152–168.

Regan, C. T. (1906a). A revision of the fishes of the South American Cichlid genera *Retroculus, Geophagus Heterogram* and *Biotoecus*. *Ann. Mag. nat. Hist.* (7), 17, 49–66.

———— (1906b). A revision of the fishes of the South American Cichlid genera *Cichla, Chaetobranchus, Chaetobranchopsis*, with notes on genera of the American Cichlidae. *Ibid.*, (7) **17**, 230–249.

———— (1913). A synopsis of the Cichlid fishes of the genus *Crenicichla*. *Ibid* (8), **11**, 498–584.

*Stannius, H. (1854). Handbuch der Anatomie der Wirbelthiere, 279, Berlin.

*Svetovidove, A. N. (1937). Uber die Klassifikation der Gadiformes oder Anacanthini (Vorlaufigei Mitteilung). *Bull. Acad. Sci. U.R.S.S.*, (Biol.), 1281.

Trewavas, E. (1933). Scientific results of the Cambridge expedition to the East African lakes, 1930–31. II. The Cichlid fishes. *J. Linn. Soc. (Zool).*, **38**, 309–341.

*Weber, E. H. (1820). De aure et auditu hominis et animalium. Pars I. De aure animalium aquatilium 134 Lipsiae.

*References marked with astoriks are not referred to in their original forms.

OSCILLATIONS AND RHYTHM IN THE VITAL ACTIVITIES OF PLANTS*[1]

by C. V. KRISHNA IYENGAR

(Communicated by T. S. Mahabalé, F.N.I.)

ABSTRACT

The author noticed oscillatory varations in the rates of several activities of plants when these were recorded at very short time-intervals. Designing suitable instruments, the author has reinvestigated and recorded the rates of Photosynthesis, Absorption of water, Transpiration, Leaf-movements, Weight changes, Respiration, Growth and Reversible Linear changes, at intervals ranging from a few minutes to a few seconds, and with magnifications of 400–6000 according to needs. Micro-changes in length, weight or volume were recorded in all these activities, and these have shown the occurence of frequent and almost regular oscillation in the rate. The duration, magnitude and frequency of these oscillations will be changing as a result of the seasonal changes, nature of the plant, its tone and age, but the manifestation of regular oscillations, or variations, in the rate seems to be an inherent feature, hence rhythmic. While the duration and frequency of the major oscillations are hours or minutes, the same for the finest oscillations will be a few seconds or fraction of a second. It is suggested that variations in pH, permeability, turgidity, enzymatic and other activities of plants, even at short intervals, may have a role in all these oscillatory changes.

The oscillations noticed in the rate of activities are also noticed in the sizes of successive plant-parts formed from time to time, and these are closely related to the annual, seasonal and intraseasonal variations in the growth conditions. The rhythmic succession of sunspot maxima, seasonal and diurnal variations are reflected in the activities of plants and animals.

From the time of its inception Life has been subjected to certain powerful influences which happen to be oscillatory and rhythmic in their nature. The periodic influence of the planets, the sunspot maxima following a certain rhythm, cosmic radiation which is more powerful than the most powerful X-ray (Sullivan, 1939), the regular succession of seasons and their peculiarities, the alternation of day and night, the diurnal variation in the intensity and quality of light and the resulting variation in the electrical charges are some of the influences to which Life had to adjust itself for its survival, development and perpetuation. Thus the rate of any activity during a period seems to be a general indication of the conditions prevailing at the time. The striking contrast in the plant's activity due to seasonal changes has left an indelible impression on the external as well as the internal structure of a plant. The general agreement between the sunspot number, weather and growth through ages has been studied and recorded by Douglass 1919), and this has enabled him to explain the climatology of several places. Thus, according to him, the plant's growth-rings are of great meteorological significance and very useful to explain the changes in weather from time to time. From his observations it is clear that there is a close relationship between the sunspot maxima and the sizes of growth-rings, and that the major and minor peaks in the maxima occur at intervals of about 23 and 11.4 years respectively. Less prominent peaks occur at intervals of a few years. The sunspot data collected by the present author have revealed oscillatory variations in the number even at intervals of a few days. Since the sunspot number and ultraviolet radiation are directly related (Stetson, 1937) it may be inferred that significant variation in the ultraviolet constituent of light seems to be a possibility if one can judge from the changing number from day to day. At times a daily variation of 20-30% in this seems to be a possibility, according to Stetson. This may be attended by variation in the electrical charges,

their reversibility and ionisation of air, thereby affecting the vital activities of plants. Since growth is the result of several factors it is not surprising that the structure of a plant is an efficient record of the weather changes and climatology of places. From the work of Douglass it is possible to understand the importance of growth-rings in the study of these changes from year to year, but the present author's work (Krishna Iyengar. 1947, 1951 and 1958) is an attempt to show that the external structure of a plant can be made use of to explain changes in growth conditions not only from season to season but also from time to time during a season.

In connection with the several vital activities of plants the author has pointed out that there are oscillatory variations in the rate, these oscillations manifesting themselves at short intervals of a few minutes or even a few seconds. The efficacy of short interval recording has been sufficiently stressed by the author in his paper on the leaf movements published in 1942. Bose, a pioneer in the field, has recorded several activities of plants at short time-intervals designing suitable instruments for his work. According to him these activities are oscillatory and rhythmic (Bose, 1923 and 1928). While there is much to be said in its favour, it must be pointed out that his observations were mostly on plants or their parts, under stimulation, hence unnatural, and so fail to give a correct picture of a plant's normal activities under natural conditions. It was this situation which prompted the author to reinvestigate most of the vital activities of plants designing simple instruments to record all these at short intervals, employing suitable magnification. The author's work on Photosynthesis, Water-Absorption, Transpiration, Respiration, Growth, Leaf-movement, Reversible changes in the weight and size of tissues (Krishna Iyengar. 1942a, 1942b, 1943, 1944, 1946b, 1946c) is an attempt to point out and establish the existence of oscillations in all these activities and explain their significance. The following is the review of the author's work.

Method of approach :

During the course of his work, the author perceived that even a simple activity like leaf-movement could furnish a clue to the plant's working mechanism, tone and daily rhythm, if only recording was done at short time-intervals, employing a high magnification. It was thus found that the Electro-Magnetic Recorder designed by the author in 1936 was inadequate on account of its low magnification. For this work the author designed an Optical Lever which could give any magnification from 400 to 6000. In combination with a sensitive balance any micro-changes in weight could be detected. A variation of 1/1000 mg could be easily recorded, while with a little of effort even a change of 1/5000 mg could be registered. The same optical lever with Float and Manometer in combination could be used to record the smallest volumetric changes. This was used by the author in his experiments on absorption, osmosis and respiration, the smallest volumetric change recorded being .000001 cc. For studying the rate of growth and linear changes in the tissues, magnifications ranging from 400 to 6000 were employed. The smallest linear change recorded during this work happens to be .0001 mm. or in other words, $1/10 \mu$. Regarding experimental details, the author's several papers give a vivid account of these. Since the author had to deal with very high magnifications in many of his experiments, it was necessary to reduce the period of observation in most of these, precautions yet being taken to maintain the external conditions almost constant during the brief periods of observations. Different experiments required different recording intervals, ranging from a few minutes to a few seconds or even less. Since the application of the 'Float and Manometer' principle was thought of after the publication of the paper on Photosynthesis (Krishna Iyengar, 1942a), it can be stated that this principle can be applied to register this activity also, thus making this method an advance over the bubbler of Wilmott (1921) and the apparatus designed by the author in 1929.

No apparatus was necessary for the study of the seasonal and intraseasonal rate of growth. The sizes of roots, root-hairs, leaves, internodes, axillary branches, flowers, fruits and seeds were measured to find out the size-variation and the resulting oscillations.

Plant's activities and oscillations :

The first attempt of the author in this line happens to be his paper on Photosynthesis published in 1942, the clue for this work being the unpublished data collected to test the working of the apparatus designed by the author in 1929. The author has shown how the rate of activity—evolution of oxygen—happens to be oscillatory in spite of the constant external conditions, and pointed out that this is due to probable fatigue, but not 'Solarisation' as explained by Ursprung (1917). A detailed enquiry has shown that significant variations in the rate generally occur at intervals of 7-11 minutes. Finer oscillations in the rate are reported to occur at even shorter intervals of a minute or more.

The next aspect studied by the author was leaf-movement (Krishna Iyengar, 1942b). In this paper an effort has been made to establish the general occurrence of the autonomic movement in all pulvinate and non-pulvinate leaves. A detailed enquiry has shown that this leaf-movement follows a daily rhythm. The oscillatory nature of these movements at long and short time intervals signified, though indirectly, the changing water-content of the plant-body. In the same paper the relationship between the leaf-movement, absorption and transpiration has been discussed in detail, and the oscillatory nature of all these activities has been pointed out. In this connection there is a reference to the oscillations in Osmosis also.

In view of the relationship between the leaf-movement and the water-content of the plant-body the author studied the weight changes in plants (Krishna Iyengar, 1946). A preliminary note was published in 1943. It was noticed that changes in weight were mostly due to the fluctuating water-content and that these were reversible, hence oscillatory, in their nature and followed a daily rhythm. The magnitude of variation in the water-content has been carefully determined, and according to the author, a daily variation of about 15-20 per cent or more seems to be a possibility in many plants, depending on their kind. The author has tried to prove that the leaf-movement is an indirect indication of the changing water-content and that the direction of movement denoted the changes towards the plus or minus side. The significance of these two aspects in the daily life of a plant has been stressed. Very fine oscillations in weight at intervals of a minute or less are also reported. According to the author, the magnitude, duration and frequency of these major and minor oscillations will depend on the kind of plant, its age, condition and several other factors, but an inherent daily rhythm always manifests itself. The author has specially stressed that a clear idea of this daily rhythm and time factor is of great importance in all investigations on the qualitative, quantitative and osmotic aspects of a plant, since different periods of the day might yield different results, in view of the significant variation in the water-content from time to time, as also the attendant variation in the rate of metabolic activities.

The fine oscillations noticed in the leaf-movements and water-content made the author to take up a critical study of the behaviour of some of the homogeneous plant-tissues (Krishna Iyengar, 1946). A detailed investigation of this aspect seemed to be necessary in view of the fact that MacDougal (1921 and 1925) and MacDougal and Shreve (1924) had reported about the rhythmic daily changes in the diameter of the stem of some plants. A note on this subject was communicated to the Indian Science Congress of 1945. For studying this aspect several kinds of tissues were carefully selected. All these manifested oscillatory linear changes when recording was done under very high magnification. Control experiments convinced the author that these changes formed a feature of the living tissues.

These oscillatory, hence reversible, linear changes were noticed at intervals of even 3-4 seconds, the duration for the finest oscillation being less than a second. The author has tried to establish that the manifestation of these oscillations is highly characteristic of all living tissues irrespective of their plant or animal origin. Thus the observations of the author form a distinct advance over those of Bose (1923), according to whom this feature is confined to a definite layer in the plant-body. However, the magnitude, duration and frequency of these oscillations may vary in the same plant, since the tone of the tissue, its age, daily and seasonal rhythm may affect them in a significant manner. These reversible changes, according to the author, denote, though indirectly, the reversibility of the several metabolic processes. Since the plant and animal tissues, especially muscles, show similar oscillations, it may be inferred that a similar working mechanism is present in all living cells. In the author's words the mechanism and its significance may be summed up as follows: 'The stimulation or depression due to variation in the intracellular concentration of CO_2, changing pH of the protoplast, and the stretching or shrinking of the protoplasm, the succeeding alteration in its structure, permeability and internal pressure are probably the successive stages preceding the linear and volumetric changes, forming thus a powerful mechanism of a living cell.' This has been styled by the author as the 'Vital Force'. The inadequacy of the physical laws to account for the high rate of water-absorption by the plant, of diffusion of CO_2 during photosynthesis, and of translocation of food materials in the plant-body, has been pointed out not only by Miller (1931) but also by many others. According to the author, it is probably this 'Vital Force' which enables absorption, elimination, translocation of materials and diffusion of gases at a rate not at all possible according to physical laws alone.

The constantly changing water-content of the plant-body in an oscillatory manner, and the intimate relationship between water-content and respiration (Bailey, & Gurjar, 1918) made the author to take up the study of respiration, designing a suitable micro-respirometer for the investigations. This apparatus works on the 'Float and Manometer' principle with the optical lever in combination (Krishna Iyengar, 1944). This has several advantages over the types designed and used by the previous workers (Osterhout & Haas, 1917, Lund, 1919, Davis, 1925, and Brown, 1942). The simple construction, high magnification, easy handling, efficient working, and lastly direct observation instead of calculation at every stage, were the points in view of the author during the construction of the apparatus. This instrument enabled the recording of even traces of oxygen taken in by a germinating seed. The oscillations in the rate of activity have been noticed even here, and these seemed to follow a time law denoting the existence of a possible rhythm. The author has pointed out that these oscillations signify the presence of alternating periods of activity and depression as a result of the changing tone of the protoplasm, and the intimately associated enzymatic action even at short intervals.

The other aspect that was tackled was growth (Krishna Iyengar, 1943b). The importance of turgor pressure, or indirectly water-content, has been stressed by Sachs (1873) and several others, and the work of the author on the leaf-movements and weight changes had already denoted the variations in the water-content even at intervals of less than a minute. In addition to these there was Friesner's work (1920) stressing the presence of a daily rhythm in mitosis and growth of plants. These were responsible for the author's attempt to reinvestigate this activity, employing high magnification and recording growth at short time-intervals. The data of Went (1925), Silberschmidt (1925) and Heyn (1931) which showed oscillations in the growth-rate but not reported by these authors strengthened the present author's conviction that the oscillations noticed in the other activities should be present in growth also in view of the inter-relationship of the vital activities. Only the aspect of linear changes was taken up for a critical study. Growth in the vegetative structures and flower buds of many plants was recorded at intervals

of 1/6 to 1/4 of a minute employing very high magnification.   In all cases oscillatory variations in the rate of growth were noticed.   The author has pointed out that the sigmoid growth-curve reported by previous workers was actually composed of series of smaller curves, the smallest curve showing a duration of a minute or less. These oscillations in the rate strongly suggest the occurrence of alternating periods of high activity and depression.   The author has hinted that ultimately the changing tone of the living matter might be responsible for these oscillations.   On account of the intimate association of the growth hormones with the protoplasm it is quite possible that the rate of formation of these as also their action are also open to similar variations, if one can judge from the significant fluctuations in the rate.

Further work on growth taken up by the author was to prove that these oscillatory variations are noticed not only in the rate of the several activities but also in the sizes of the different plant-parts as also the development of these.   In this work, the seasonal and intraseasonal variation in the sizes of leaves and internodes was studied in detail, and a paper was communicated to the Indian Science Congress of 1946.   An attempt has been made to show that the sizes of the successive leaves and internodes formed during any season show a similar oscillatory nature and to explain the relationship between these size variations and the seasonal conditions of a place.   According to the author, the seasonal and intraseasonal peculiarities are generally reflected in the varying sizes of the plant-parts, making the annual or seasonal branch an efficient record of not only growth-conditions but also, though indirectly, the weather changes (Krishna Iyengar, 1947 and unpublished).   The author has also reported that there is a similar variation in the other parts of a plant also. Thus, according to him, not only the vegetative but also the reproductive parts manifest this feature, and the successive leaves, internodes, flowers or fruits formed during a season show parabolic size variation, the best parts being formed about the middle of a seasonal growth.   Even in a fruit there is a similar parabolic variation in the sizes of the successive seeds, the best seeds being placed about the middle. The general parabolic curve of the season shows finer oscillations when the number of structures formed during a season happens to be large.   Even a leaf shows the parabolically varying rates of growth along its length during its development, the middle part of a leaf generally showing the maximum rate of growth.   The variation in the sizes may be taken to denote the varying vigour and fertility of the successive parts.   According to the author, the position of the part on the axis would determine the advantages or disadvantages in its development and expression.   Since the size and vigour are generally determined by position, an intimate knowledge of this biological principle is strongly recommended by the author in all investigations of the Biological Sciences.   The author's elaborate work during the last several years (Krishna Iyengar, 1947, and unpublished) is an attempt to show its importance in propagation and cultivation of plants, and in the selection of suitable seed-material. It has been shown to be possible not only to increase the yield but also to improve its quality.   A similar seasonal and intra-seasonal variation is noticed in the activities of animals also.   According to the author the highly significant and constantly present parabolic variation in size, vigour and fertility may denote the varying hormonic, enzymatic and other activities of not only the entire plants but also their parts.

It is thus noticed that all activities of plants are oscillatory.   A diagram is introduced below to illustrate these and their relationship.   The plant's growth through ages as recorded by Douglass shows the almost regular oscillations, these generally agreeing with the sunspot cycle and manifesting highly pronounced peaks at intervals of 11.4 and 23 years (Fig. A).   It is noticed that each of these curves is composed of minor oscillations, the duration of each of these being about 3 years or slightly more.   When the annual growth is considered there are two major peaks in the rate of growth.   Although this has been the general feature as explained by Sachs and others, a departure from this in the form of two more, though minor,
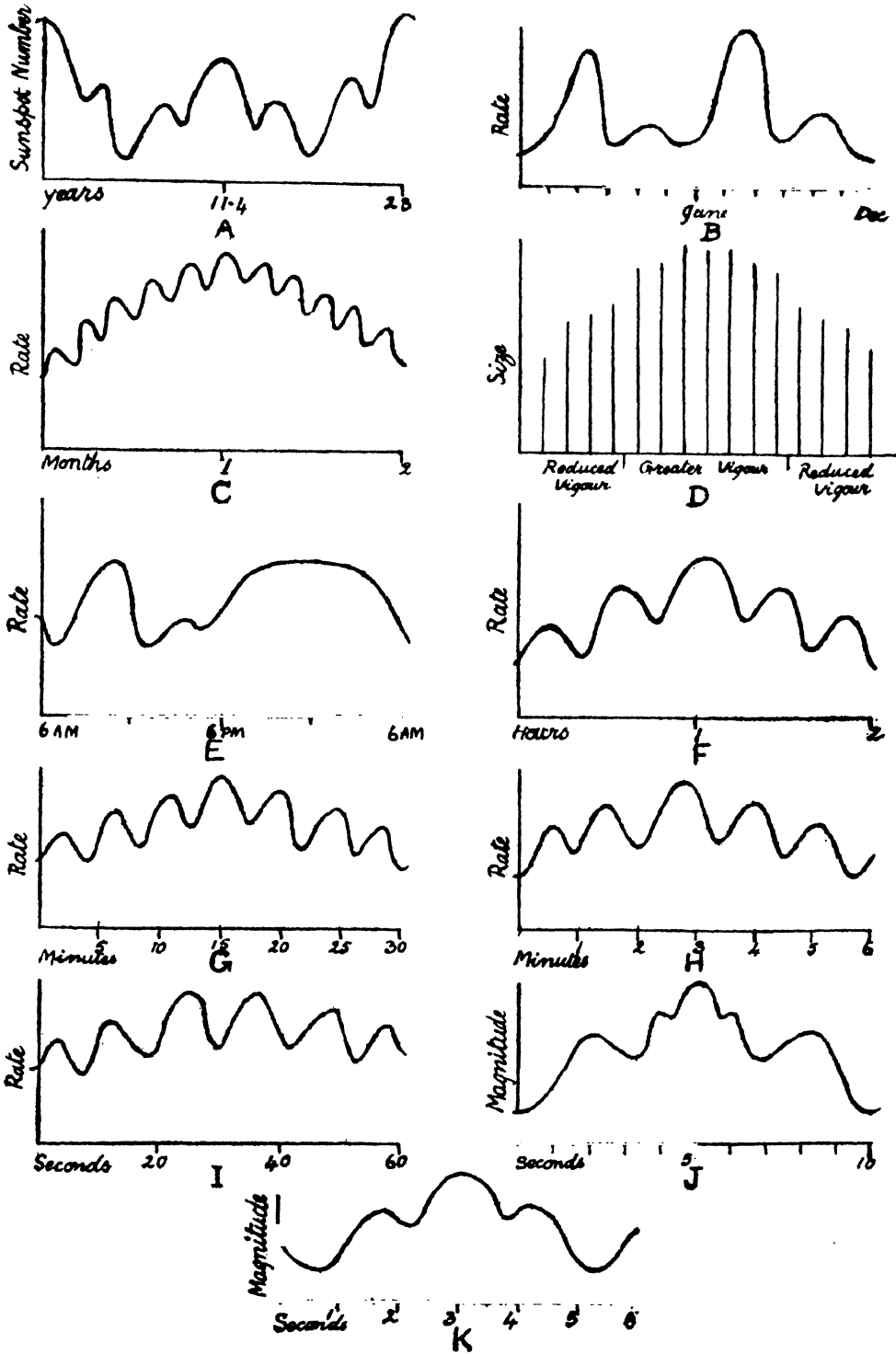
TEXT-FIG. 1.

Chart showing the several types of oscillations met with in plant's activities.
Explanation in the text.

peaks alternating with these major peaks has been reported by the author, in the paper published in 1947, these denoting the peculiarties in the growth-conditions and climate in certain parts of India (Fig. B). Intraseasonal growth-rate also shows similar oscillations (Fig. C). In this connection may be mentioned the parabolic variation in the sizes of the parts, their development and vigour, as also fertility, in any seasonal growth (Fig. D). Even the daily record of the sunspot numbers during a season shows a similar oscillatory nature. The daily activity seems to be a miniature form of the annual activity, the two pronounced peaks in the rate of growth noticed even here heightening the similarity (Fig. E). This has been noticed not only in growth but also in the water-content and in the rate of photosynthesis (Mclean 1920). Even in the leaf-movement a similar feature has been reported by the author. The figure F is introduced to show how an oscillation taking an hour or more is composed of minor oscillations, the duration of each of these being 20-25 minutes. This has been explained by the author in several activities of plants. Each of these oscillations is made up of several finer oscillations the time taken for each one of these being generally 3-5 minutes. The Figure G illustrates this feature. These have been explained by the author in connection with respiration, growth and other activities. Even finer oscillations, each of about a minute's duration, have been shown to be present in several activities (Fig. H). According to the author, these are composed of still finer oscillations, each taking 6-10 seconds (Fig. I). The finest oscillations are reported by the author in connection with the tissue study, the time taken for each being 3-4 seconds (Fig. J). The author has noticed even finer oscillations of a second's duration or less (Fig. K), but it has not been possible for him to give a clear account of these, since a magnification of even 6000 seemed to be inadequate for this work. The author is convinced that a higher magnification and a shorter recording interval might enable one to perceive the finest oscillations and study their nature. Such high magnifications have often been used in the study of the nervous impulses, but in such work, instead of linear changes potential variations at intervals of micro-fractions of a second are registered with the help of oscillograph. In some cases the frequency of these oscillations happens to be about 1000 or more per second. This electrical change even at such short intervals has been said to be due to the changing pH of the living cell. The use of such instruments may be thought of to determine the presence or absence of similar oscillations in plants.

Thus the occurrence of oscillations in all the vital activities of plants and animals is quite a common feature, these suggesting the presence of alternating periods of activity and depression as a result of the changing tone of the protoplasm. Since various enzymes are associated with the protoplasm for carrying on the several activities, the oscillatory changes in the living matter might signify a corresponding variation in the enzymatic action and the metabolic processes. This has been stressed by the author in connection with the tissue studies. According to the author, this might mean a rapid reversibility in the pH of the protoplast followed by an equally rapid change in the enzymatic action. Thus, the protoplasm, the activated organic matter, exhibits an oscillatory nature in the rates of both anabolic and catabolic processes, making one infer that the absorption or liberation of energy by the activated matter is always oscillatory. This should not come to one as a surprise, in view of the fact that even inanimate matter shows a similar feature when it is activated. As an instance may be cited radium which emanates rays whose intensity is reported to be oscillatory (Moller and Ebbe Rasmussen, 1937). Leading scientists are of opinion that the absorption or liberation of energy by any activated atom is always in 'quanta', the rate of activity thus being oscillatory. This illustrates the similarity between the activity of the living and the activated non-living matter, making the difference between the 'Living' and the 'Non-living' more subtle.

Rhythm in Life :

The work reviewed above shows that all activities are oscillatory, and follow a definite time-law exhibiting not only periodicity but also an inherent rhythm. For a long time 'rhythm' and 'periodicity' were the terms used almost as synonyms. Sachs (1875) and others were the earliest to use the term 'rhythm' in this sense. But it may be pointed out that 'periodicity' is the result of external influence while 'rhythm' is a regular periodic change not at all connected with the external factors. Schimper (1891), Friesner (1920) and Macgregor Skene (1932) have a correct conception of these terms. Macgregor Skene's statement 'we must note that the production of leaves is itself a periodic phenomenon, as is, to go a step further back, cell divion also, the basic fact in development, and that the cause of such a rhythm must be sought in periodic changes inherent in the complex colloids of the plasma' is highly suggestive of the existence of an inherent rhythm, which could be disturbed, According to Macgregor Skene only by methods unnatural and drastic, thus being a powerful inherent feature. The sprouting and flowering of many plants only during certain months of the year is an instance of this kind. According to Bose (1928) the responsive movements are also rhythmic. The rhythm noticed in several activities has been sufficiently stressed by the author. Even in the lower plants one can notice this feature in their several activities. The ciliary movements of the zoospores and gametes, and the size variation of the contractile vacuoles at regular intervals are a few of the instances of this feature met with in the lower plants and animals. The systole and the diastole of the heart, the respiratory action and the regularly alternating activity and fatigue are a few instances of rhythm noticed in animals. The variation in pH, and the succeeding oscillations in metabolic activity following a daily rhythm, vigour and fertility satisfying a definite time law, oscillatory metabolic variations even at very short intervals of a fraction of a second, and lastly the regularly alternating rise and fall in the rate of any vital activity, seem to be a feature of plants and animals alike.

From the above account it can be inferred that oscillation in any activity seems to be the rule in Nature, and that the only constant thing in Nature happens to be the capacity to vary. The regular succession of sunspot maxima, of seasons and seasonal changes, and of day and night are clearly reflected in the activities of not only plants but also animals. Phase of dormancy and activity, growth and reproduction, sleep and wakefulness, assimilation and dissimilation, and finally activity and fatigue—the contrasting processes in Life—alternating in a regular, hence rhythmic manner, convey a vivid picture of Life's rhythm forming, though in miniature, a reflection of the same in Nature.

ACKNOWLEDGEMENTS

REFERENCES

Bailey, C. H. and Gurjar, A. M. (1918). Respiration in stored wheat. *J. agric. Res.*, **12**, 685–713.
Bose, J. C. (1913). On diurnal variation in the Meto-exitability in *Mimosa*. *Ann. Bot. Lond.*, **27**, 759–79.
———— (1923). Physiology of the Ascent of Sap. London.
———— (1928). Motor Mechanism of Plants. London.

Brown, R. (1942). The gaseous exchange of seeds and isolated cotyledons of Cucurbita pepo. *Ann. Bot., Lond.*, **6**, 293–321.

Davis, W. E. (1925). A simple and rapid method of studying respiration by the detection of exceedingly minute quantities of Carbon dioxide. *Science, N.S.*, **44**, 105–108.

Douglass, A. E. (1919). Climatic cycles and tree-growth. *Carnegie Inst. Publ.*, Washington.

Friesner, R. C. (1920). Daily rhythm of elongation of, and cell division in certain roots. *Amer. J. Bot.*, **7**, 380–406.

Heyn, A. N. J. (1931). Der Mechanismus der Zellstrechung. *Rec. Trav. Bot. néerl.*, **28**, 113–244.

C. V. Krishna Iyengar (1929). Apparatus to measure the photosynthetic activity in some of the water plants. *Half-yrly J. Mysore Univ.*, **1**, 1–7.

— — — (1936). A new type of electric recorder for plant autographs. *J. Indian bot. Soc.*, **15**, 175–177.

— — — (1942a). Variation in the photosynthetic rate in *Elodea*, *Ibid.*, **21**, 167–171.

— — — (1942b). Autonomic movements of leaves and their relationship to the water-content of the plant. *Half-yrly J. Mysore Univ.*, **3**, 23–38.

— — — (1943a). Fluctuation in the weight of a plant, *Curr. Sci.*, **12**, 188–189.

— — — (1943b). Variation in the rate of growth in plants, *Proc. nat. Inst. Sci. India*, **9**, 351–356.

— — — (1944). Variation in the rate of respiration of a germinating seed. *J. Indian bot. Soc.*, **23**, 9–20.

— — — (1945). Reversible linear changes in the tissues of plants. *Proc. Indian Sci. Congr.*, Part III, Section 8.

— — — (1946a). Intraseasonal variation in the sizes of leaves and internodes. *Ibid.*, Part III, Section 6.

— — — (1946b). Reversible changes in the weight of a plant. *J. Indian bot. Soc.*, **25**, 151–161.

— — — (1946c). Reversible linear changes in the tissues of plants and their significance. *Proc. nat. Inst. Sci. India*, **12**, 267–275.

— — — (1947). Intraseasonal variation in growth and propagation of plants. *J. Indian bot. Soc.*, **26**, 143–156.

— — — (1951). Intraseasonal Growth-Variation and cultivation of Sugar-cane. *Nature, Lond.*, 168.

— — — Some aspects of Intraseasonal Growth-Variation in plants. Unpublished.

Lund, E. J. (1919). A simple method of measuring Carbon dioxide produced by small organisms, *Biol. Bull.*, **36**, 105–114.

MacDougal, D. T. (1921). Growth in trees, *Carnegie Inst. Washington Pub.*, 365.

— — — (1925). Reversible Variation in Volume, Pressure and Movement of Sap. in Trees. *Ibid.*, 365.

MacDougal, D. T. and Shreve, F. (1924). Growth in trees and massive organs of plants. *Ibid.*, 350.

Macgregor Skene, (1932) Biology of the flowering plants. 2nd revised imp. London.

Mclean, F. T. (1920). Field studies of the Carbon dioxide absorption of coconut leaves. *Ann. Bot., Lond.* **34**, 367.

Miller, E. C. (1931). Plant Physiology. New York.

Moller, C. and Ebbe Rasmussen. (1939). World and the Atom. London.

Osterhout, W. J. V. and Haas, A. R. C. (1917). An adaptation of Winkler's method to biological work. *J. biol. Chem.*, **32**, 141–146.

Sachs, J. (1873). Über das Wachstum der Haupt und Nebenwurzeln. *Arb. bot. Inst. Wurzburg.*, **1**, 385–474.

Schimper, A. F. W. (1891). Die Indo-Malayische Strant Flora. Jena.

Silberschmidt, K. (1925). Untersuchungen über die Thermowachstumsreaktion. *Ber. dtsch. Bot. Ges.*, **43**, 475–482.

Stetson, H. T. (1937). Sunspots and their effects. London.

Ursprung A. (1917). Über die Starkebildung in Spektrum. *Ber. dtsch. Bot. Ges.*, **35**.

Went, F. W. (1925). Concerning the difference in sensibility of the tip and base of *Avena* to light. *Proc. Acad. Sci. Amst.*, **29** 185–191.

Wilmott A. J. (1921). Experimental researches on vegetable assimilation and respiration XIV. Assimilation by submerged plants in dilute solutions of Bicarbonate and Acids. An Improved Bubble counting Technique. *Proc. roy. Soc.*, (B)**92**, 304–327.

STRUCTURAL MODIFICATION OF THE CLOACA OF *LYCODON AULICUS AULICUS*, LINN., IN RELATION TO URINE EXCRETION AND THE PRESENCE OF SEXUAL SEGMENT IN THE KIDNEY OF MALE*

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(Communicated by M. L. Bhatia, F.N.I.)

ABSTRACT

Morphology of cloaca of *Lycodon aulicus* has been described. Coprodaeum is muscular and is divided into five compartments, each chamber invariably contains a small rounded pellet of urine. The division of coprodaeum into a number of compartments, in all probability checks the formation of a long, hard urinary pellet which may prove a great hindrance during locomotion. Uric acid forms 72 per cent of the excretory nitrogen.

INTRODUCTION

Bodenheimer (1957) in a review on the "Ecology of mammals in arid zones" states that, "In view of the extremely low total on the heavy exigencies of the very dry desert environments there must be some extraordinary ecological and/or physiological adaptation for a conservation of body water. Highly concentrated urine is an important mechanism for such a water economy, by reducing the total amount of urine water to be excreted to a minimum". If during the period of particular stress on the water conservation mechanism, animals could avoid the use of water for excretion of substances usually eliminated in the urine, a corresponding amount of water would be saved. This mechanism is known to occur in insects, where it is termed 'Storage excretion'. The most important nitrogenous product is uric acid and it is regularly deposited in cells of the fat body instead of being eliminated in the urine. This phenomenon of uric acid excretion is predominant in lizards and snakes. In these animals it cannot be termed 'Storage excretion' because they are not stored in cells, but on the other hand urine gets dehydrated by the reabsorption of water and solid urine is excreted.

Observations on the water economy in relation to urine excretion of a few lizards have already been presented in previous papers. The present paper deals with a similar phenomenon in the common wolf-snake, *Lycodon aulicus aulicus*. The method for the collection of solid urine and the technique for the chemical analysis are similar to those described earlier (Seshadri, 1956 *a*, *b*, 1957).

MORPHOLOGY

The most interesting point in the morphology of the urinary system of snakes is the total absence of urinary bladder. There are certain lizards which are classified as saurii B by Gadow which include the Monitors, Amphisbaenids and Agamids in which the urinary bladder is absent.

The urinary system in *Lycodon aulicus* consists of a pair of kidneys, two ureters, one arising from each kidney, and a chambered cloaca into which the ureters and the genital ducts open. Because of the longish body, in a snake all the organs

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in its visceral cavity are elongated and narrow. The kidneys and also the cloaca are elongated. The kidney in snakes and lizards is of special interest because of the unusual secretory activity of the segment preterminal. The kidneys lie dorsal to the cloaca and each consists of five indistinct lobes. A peculiar feature in the kidney is that although it is a compact body its tubules can be easily separated with the dissecting needles by following Huber's method. There is a slight morphological difference in the kidneys of the two sexes in *Lycodon*. On the dorsal side of the kidney of male are thick, rounded tubules embedded among the finer tubules, and on the ventral side finer tubules are present, large tubules are confined only to the outer side of the ureter (Fig. 1). In the kidney of female, however, the tubules on the dorsal and ventral side are small and almost alike, and kidneys are richly supplied with blood. In a freshly dissected specimen ventral side of the kidney is deep red in colour, while on the dorsal side, specially in the male, yellow patches are present on the large kidney tubules.

The ureter from each kidney emerges just a little behind one third of the kidney proper and extends along and just within the ventro-median angle of the kidney near its posterior end. Actually the ureter in each kidney starts much ahead, as is seen in sections, where the collecting tubules join to form the ureter proper.

The cloaca as usual is divided into an anterior chamber, the coprodaeum, an intermediate chamber, the urodaeum and a posterior chamber, the proctodaeum. A sphincter separates coprodaeum from urodaeum and there is only a slight demarcation of proctodaeum from the urodaeum. The coprodaeum is a continuation of the large intestine but it is demarcated from the rectum by a transverse fold that hangs from the dorsal wall. The coprodaeum is the largest of the three chambers, it is half as long as rectum and about five times as large as urodaeum. This chamber is further divided into as many as five compartments by incomplete transverse folds, more prominent on the dorsal side of the chamber (Fig. 1). In several dissections of the cloacal portion almost all the compartments of coprodaeum had a small, rounded urinary pellet in the process of formation into a large pellet. When solid urine is finally evacuated in the natural course it is always in the form of a single, large; elongated pellet which has a beaded appearance. This shape is due to the pellet of the different compartments uniting together before they are actually evacuated. In some specimens, in the last two coprodaeal compartments the pellets were observed in the process of formation. In such cases a well formed rounded pellet was not seen and a number of small crystalline pieces were present instead, which at a later stage unite, become compact and form a rounded pellet.

Urodaeum or the urinogenital sinus is the next chamber of thee loaca into which both the urinary and the genital ducts open. This chamber in natural course is placed slightly on the dorsal side of the coprodaeum which gives the impression that coprodaeum is more or less in direct communication with the proctodaeum. In male *Lycodon* the ureter and the vas deferens join before they open in a common urinogenital aperture. In female *Lycodon* the urodaeum is functionally divided into a large anterior genital sinus and small posterior urinary sinus. Both the ureters unite to form a small pseudo bladder-like dilatation and open on a papilla in the dorso-median line. In female *Calotes* there seems to exist a similar structure and it has also been recorded in *Lophura* (lizard) by Gadow (1887). The coprodacal chamber is placed ventrally, and such an arrangement presumably facilitates an easy flow of liquid urine into the coprodaeum where it undergoes dehydration.

The oviducts open on lateral sides of the genital sinus or genital shelf, which extends dorsally up to the level of the anterior portion of coprodaeum. Passage to the opening of the oviducts from the proctodaeum is on either side of the urinary sinus or sac. Lining of the genital sinus resolves into a number of longitudinal folds which continue back and merge into the folds of proctodaeum.

Proctodaeum is not demarcated from urodaeum by any fold or sphincter but the position of urinary aperture seems to indicate the boundary of the two chambers.

In male *Lycodon* there are two grooves in proctodaeum and on either side of proctodaeum, more towards the dorsal side, are a pair of glands, the anal glands which seem to lead into the grooves.
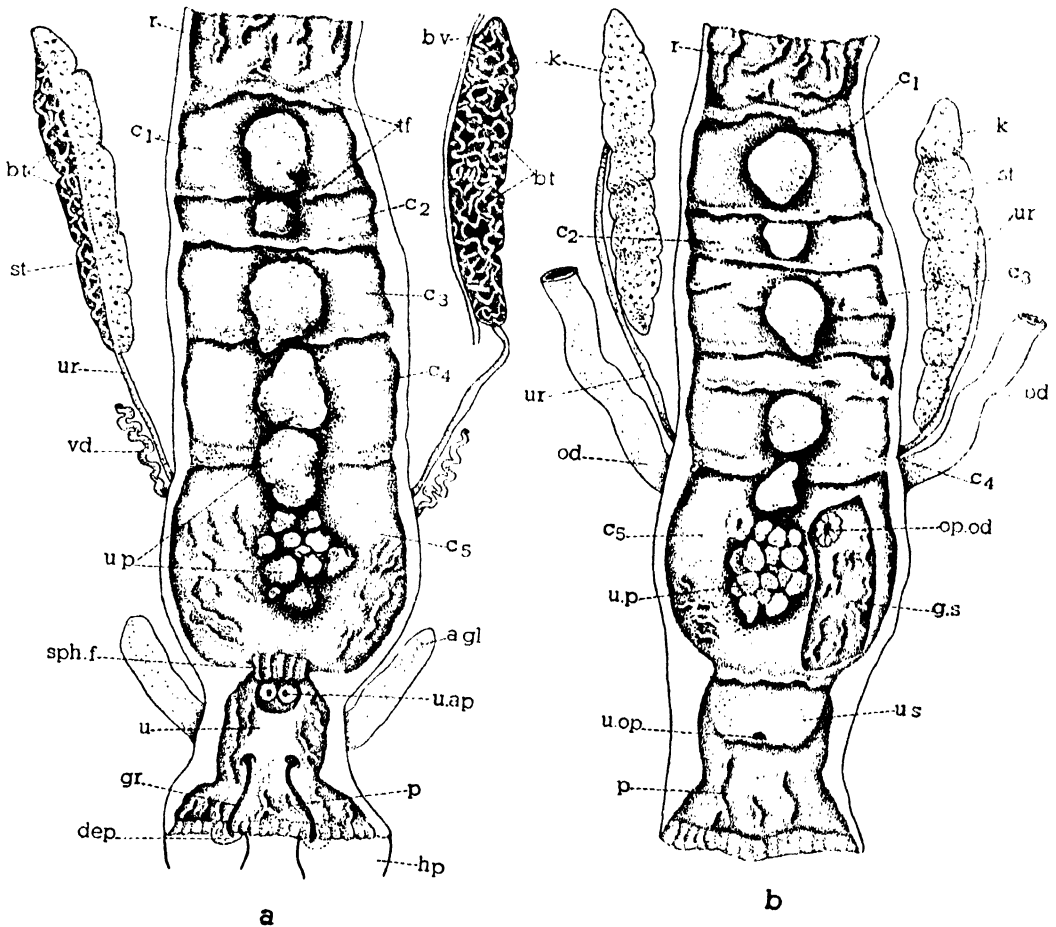
TEXT-FIG. 1.

Lycodon aulicus aulicus. Dissection of the urinogenital systems of male and female. *a. gl.*, anal gland; *bv.*, blood vessel; *b.t.*, big tubules on the dorsal surface of kidney; *c1–5*, compartments of the coprodaeum; *dep.*, depression at the place where hemipenis comes out; *gr.*, groove leading to the hemipenis; *g. s.*, genital sinus; *hp.*, hemipenis; *k.*, kidney; *od.*, oviduct; *op. od.*, opening of oviduct; *p.*, proctodaeum; *r.*, rectum; *s.t.*, small tubules of kidney; *sph. f.*, longitudinal folds in the region of the sphincter; *t. f.*, transverse folds dividing coprodaeum into chambers; *u. ap.*, urinogenital aperture; *u. op.*, urinary opening; *u. p.*, urinary pellets; *ur.*, ureter; *u. s.*, urinary sinus; *vd.*, vas deferens.

HISTOLOGY

Most conspicuous feature in the histology of kidney is the presence of relatively large segments of renal tubules (Fig. 2c).   Such a segment was first described by Gampert (1866) in *Tropidonotus natrix*.   While Regaud and Policard (1903*a, b, c*) first described and named in lizard a remarkable specialization and hypertrophy of the 'segment preterminal' of the nephron of adult male during the breeding

season. These authors referred to the enlarged tubule as the 'segment sexuel' and its sexual significance was also recognised by them in snakes. It has since been described by several other workers and such hypertrophy is reported to appear only in adult males and is obviously a secondary sex character.

A sexual segment has also been reported in the kidney of many snakes : *Tropidonotus natrix* (Gampert, 1866; Heidenhain, 1874; Regaud and Policard, 1903 *a, b, c*; Cordier, 1928), *T. viperinus* (*Natrix naura*) (Regaud and Policard, Cordier), *Entacuia sirtalis, Coronella austriaca, Zamenis viridiflavous. Vipera aspis* and *V. berus*.

Regaud and Policard have summarized data relevant to the morphology of urinary tubule of snakes. They divide the tubule into six parts : (1) a short ciliated or flagellated neck, opening into the Bowman's capsule; (2) a convoluted segment homologous to convoluted tubule of the mammal; (3) an intermediate ciliated segment which probably corresponds to Henle's loop; (4) a narrow non-ciliated segment; (5) a preterminal segment, part of which is the sexual segment; and (6) a short terminal segment emptying into a collecting duct.

According to Regaud and Policard and others the 'segment sexuel' is the segment preterminal because it is said to be succeeded by a short terminal tubule, which in time empties into a collecting duct. Forbes is convinced that the hypertrophied renal tubule of *Sceloporus* is neither next to the last (Preterminal) segment of the nephron nor the terminal segment of the nephron. He believes that hypertrophied tubules are the collecting tubules. But in *Lycodon* there are other collecting tubules as well, so it can be called the segment preterminal.

The mucosal cells of the sexual 'segment' comprise of very tall, simple, columnar epithelium supported by a thin layer of connective tissue. Each cell has an irregular, strongly basophilic nucleus at the extreme peripheral end. The inner two thirds of cytoplasm contains large number of markedly eosinophilic secretion granules, these tubules therefore take very dark eosin stain while other tubules do not. In spite of large size of these tubules, the lumen of each tubule is not large, while a collecting tubule has cuboidal cells and a large lumen. The tubules are not eosinophilic in character. The collecting tubules and the sexual 'segment' can be easily distinguished by these two primary characters.

At the height of its secretory activity the sexual segment in male snake is approximately three times the diameter of other tubules. In female such an enlargement is not present in the tubules It is an established fact (Regaud and Policard) that seasonal variations occur in sexual segment of many male lizards. There has been some question, however, as to whether they are also characteristic of snakes. Cordier (1928) found evidences of secretion but no cyclic variation from May to December. Volsoe (1944) was the first to demonstrate total absence of significant annual fluctuations in the height of cells and in the cytological indication of secretion. In *Lycodon aulicus* very high secretory activity was observed by the author in the month of October. The secretory granules get stained with eosin and nuclei dark blue with haematoxylin. they are basal in position and irregular in outline.

The mucosa of coprodaeum consists of long, columnar cells (Fig. 2*a*). It is interesting to note that these cells gradually increase in size and are largest in the last compartment of coprodaeum, which indicates that the terminal chamber is capable of absorbing maximum amount of water. This view is also strongly supported by the fact that a number of small crystals were every time observed in this region and pellets in the process of formation. As pellet gets formed it is pushed into the adjoining compartments in front till it reaches the first where its further forward movement is checked by the thick transverse fold of the rectum. Pellet is pushed from behind forwards by essentially an antiperistaltic movement. The wall of coprodaeum is highly muscular as compared to that of the lizards studied in this connection. This may facilitate movement of pellets up and down the large copro-
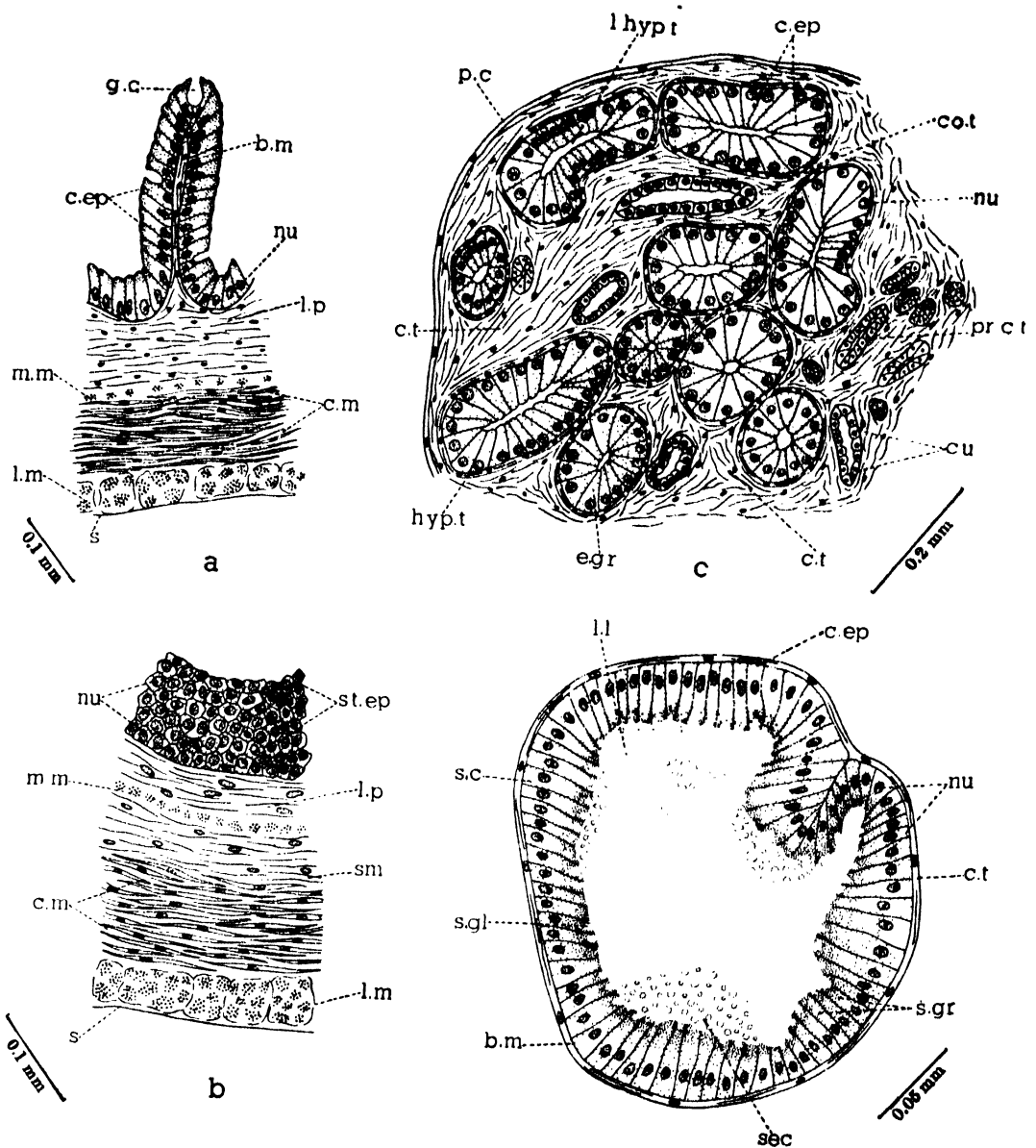
TEXT-FIG. 2.

Lycodon aulicus aulicus. (a) Transverse section of a portion of coprodaeum, (b) transverse section of the wall of urodaeum, (c) transverse section of a portion of a portion kidney showing hypertrophied tubules, and (d) transverse section of a lobule of anal gland showing excretory meterial.

b. m., basement membrane; *c. ep.*, columnar epithelium; *c. m.*, circular layer of muscles; *c. t.*, connective tissue layer; *co. t.*, collecting tubule; *cu.*, cuboidal cell of collecting tubule; *e. gr.*, eosinophilic granules; *g. c.*, goblet cells; *hyp. t.*, hypertrophied tubule; *l. hypt.*, lumen of hypertrophied tubule; *l. l.*, lumen of lobule; *l. m.*, longitudinal layer of muscles; *l. p.*, lamina propria; *m. m.*, muscularis mucosa; *nu.*, nucleus; *p. c.*, peritoneal covering; *pr., c. t.*, proximal convoluted tubule; *s.*, serosa; *s. c.*, secretory cell; *sec.*, secretory material; *s. gl.*, secretory globule; *sm.*, submucosa; *st. ep.*, stratified epithelium.

daeal chamber. In lizards, where this chamber is not so large, a thick wall is not necessary to push the urine. Peristaltic movements of rectum which ordinarily push the faeces also serve to evacuate the solid urine. But in *Lycodon*, as already indicated, the chamber as a whole is very large, divided into compartments, and its wall is muscular, which also facilitates evacuation of urine

Urodaeum has several layers of stratified epithelial cells (Fig. 2b), the genital shelf in particular shows intense stratification while the urinary sac has the usual cuboidal cells similar to those of the ureter. The stratification extends behind to the proctodaeum and finally merges with the stratified squamous epithelium of the integumental epidermis at the anal margin. In male *Lycodon* small isolated areas of cuboidal epithelium are occasionally interspersed behind the urinogenital aperture in the stratified mucosa.

Anal glands are present both in male and female *Lycodon*. In male these glands are much bigger than in female. The gland is mucous secreting and in cross-section secretory material is seen in the lumen of the lobules (Fig. 2d). The gland as a whole is made up of a number of tubular units, which in cross-section are cut very much like slices of a pie. The outermost layer of the duct is the connective tissue layer which is very thin. Next to it is the basement membrane on which rests the cellular epithelium. The secretory cells are more or less columnar in shape, each cell having a basal nucleus. The cytoplasm is filled with secretory granules and in some cells secretory globules are observed at the ental tip.

DISCUSSION

Gadow (1887) remarked that anal glands of female ophidian are much larger than those of male and that they occupy the whole space otherwise occupied by the male organ. In *Lycodon*, however, it has been observed that large anal glands are present in male, and they are much smaller in female. Even in *Coluber ventromaculatus* the anal glands in male are well developed and there are prominent proctodaeal grooves.

The division of coprodaeum into a number of compartments must have some functional significance. Though no experiment has been performed to ascertain the actual working of coprodaeum it may be possible to conjecture on the basis of morphological studies. To facilitate serpentine movement, all the organs in the body cavity of a snake are elongated and are pliable by nature. Urine gets dehydrated in the coprodaeal chamber. If such a long chamber was without compartments, a fully formed, long urinary pellet in it will hinder the activities of the animal in many ways. This is specially so when active locomotion is taken into consideration. A long, hard urinary pellet in the coprodaeum would make the chamber rigid and will by no means be a very convenient structure and might rupture the wall or injure it during locomotion. Small pellets in different compartments maintain flexibility of the chamber as a whole, when peristalsis starts to evacuate the faeces, the faecal matter as it travels down pushes the urinary pellets as well. In this act all the small pellets get stuck together one over the other and finally when the urine is evacuated it is in the form of a single long urinary pellet. The vertebrae in this region are small, and each vertebra is more or less the size of a single compartment of the coprodaeum. This also supports the above view that the division of coprodaeum into compartments helps in flexibility of the chamber. All this is at best just a conjecture without any experimental evidence.

Results of chemical analysis of urine:

Constituents			Percentage per gm. of urine
Urea	..	..	Traces
Creatine	..	..	3.4%
Creatinine	..	..	Traces
Uric acid	..	..	72%
Allantoin	..	..	1.1%
Ash	..	..	11.0%

Some of the urinary nitrogen constituents have been determined in a few snakes. Boussingault (1950) found 80 per cent of excretory nitrogen of boa constrictor and of python in the form of uric acid. Bacon (1909) claims that uric acid in python amounts to 89 per cent. Girod (1892) working on *Tropidonotus natrix*, reports that uric acid represents 80 per cent of the excretory nitrogen. Fouad Khalil (1948 *a*, *b*) worked on an oviparous snake *Zamenis diadema* and on a viviparous snake *Eryx thebaicus*. In *Zamenis* he records 67 per cent of uric acid and in *Eryx* uric acid represents 62.5 per cent of the total excretory nitrogen. Value for uric acid obtained by Khalil is much lower than that obtained by earlier workers. In *Lycodon aulicus* an oviparous snake uric acid represents 72 per cent of the total excretory nitrogen. This seems to give an intermediate value.

Percentage of creatine in *Lycodon* is high against what Khalil has recorded in *Eryx* and *Zamenis* which seem to be negligible quantities i.e., 0.20 and 0.06 per cent respectively. In *Lycodon* it is 3.4 per cent. Percentage of urea as recorded by Khalil in *Zamenis* is 2.03 and in *Eryx* it is absent. In *Lycodon* urea is present in small traces.

REFERENCES

Bodenheimer, F. S. (1957). The ecology of mammals in arid zones. Arid Zone Research—VIII, Human and animal ecology, Reviews of Research.

Cordier, R. (1928). Études histophysiologiques sur le tube urinaire des reptiles. *Arch. Biol., Paris*, **38**, 111-171.

Gadow, H. (1887). Remarks on the cloaca and copulatory organs of the Amniota. *Phil. Trans.*, B**178**, 5-37.

Herlant, H. (1933). Recherches histologiques et experimentales sur les variations cycliques du testicule et des caracteres sexuels secondaries chez les reptiles. *Arch. Biol., Paris*, **44**, 347-468.

Huber, G. C. (1906). The morphology of the urinary tubules of the reptilian kidney. *Brit. med. J.*, No. 2, pp. 1701.

———— (1917). On the morphology of the renal tubules of vertebrates. *Anat. Rec.*, **13**, 305-399.

Khalil, F. (1948*a*). Excretion in reptiles. II. Nitrogen constituents of the urinary concretions of the oviparious snake, *Zamenis diadema*, Schlegel. *J. biol. Chem.*, **172**,(1), 101-103.

———— (1948*b*). Excretion in reptiles. III. Nitrogen constituents of the urinary concretions of the viviparious snake, *Eryx thebiacus* Reuss. *Ibid*, **172**,(1), 105-106.

Regaud, C. and Policard, A. (1903*a*). Variations sexuelles de structure dans le segment preterminal du tube urinifere de quelques ophidiens. *C. R. Soc. Biol., Paris*, **55**, 216-218.

———— (1903*b*). Recherches sur la structure du rein de quelques ophidiens. *Arch. Anat. micr.*, pp. 191-282.

———— (1903*c*). Sur les variation sexuelles de structure dans le rein des reptiles *C. R. Soc. Biol., Paris*, **55**, 973-974.

Seshadri, C. (1956a).   Urinary excretion in the Indian House lizard, *Hemidactylus flaviviridis* (Ruppell).   *J. zool. Soc. India*, **8**, 63–78.

———— (1956b).   Urinogenital organs and urinary excretion in the pond turtle, *Lisemys punctata punctata* Bonnaterre.   *Ibid.*, **8**, 197–210.

———— (1957).   Water conservation in *Uromastix hardwickii* (Gray) and the presence of Müllerian duct in the male.   *Ibid.*, **9** (2), 103–113.

———— (1959).   Functional morphology of the cloaca of *Varanus monitor* (Linnaeus) in relation to water economy.   *Proc. nat. Inst. Sci. India*, **25** B(2), 101–106.

Volsoe, H. (1944).   Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L).   Reprinted from the *Spolia Zool. Mus. Hauniensis* Bianco Lunos Bogtrykkeri a/s Copenhagen, Vol. **172**.

DEVELOPMENTAL STUDIES : IV. THE ORIGIN AND VASCULARIZATION OF THE STIPULES AND AXILLARY BUDS OF *GARDENIA, PORTLANDIA, RANDIA, MUSSAENDA, LUCULIA, COFFEA, ADINA, MORINDA, HYMENODICTYON, HAMILTONIA* AND *RUBIA**

by PRADYOTKUMAR PAL**, *Department of Agriculture, Calcutta University*

(Communicated by G. P. Majumdar, F.N.I.)

ABSTRACT

The origin and vascularization of the stipules in 13 species, and those of the axillary buds in 24 species of the Rubiaceae have been reported in this paper. In 21 species the leaves are opposite, and only in 3 they are whorled.

At the initiation of a leaf at the shoot apex the first part to be laid down is the *leaf-base*, which at this stage is confluent with the axis. Two or three such leaf-bases at the node unite by their margins and form a *collar* round the axis. This collar grows with the axis for some time and then separate from the latter in the form of a tubular sheath. The stipules, *connate*, *interpetiolar* and *foliaceous*, develop as outgrowths of the collar after the petioles have separated from it. The stipules get their vascular supply (stipular trace) from the lateral leaf-trace bundles and/or their branches.

In the Rubiaceae the axillary buds are both *foliar* and *axial* in origin, and they occur in the same bud, sometimes at different nodes, in some cases at the same node in the axil of the same leaf. In almost all the species the foliar buds originate near the apex of the parent bud, and are ephemeral. Whereas the axial buds which develop into branches arise a few nodes below the apex and get their vascular supply directly from the central stele. On an adult axis there are always branchless nodes between two nodes with branches. The morphology of the axillary bud and its relationship with its axillant leaf have been discussed.

INTRODUCTION

This is a further contribution on the results of a systematic study of the origin, vascularization and morphology of the stipules and axillary buds of the Rubiaceae. Methods of studies followed are the same as in previous communications (Majumdar and Pal, 1958*a*, 1958*b*, 1959), but the materials are different.

OBSERVATIONS

1. *Gardenia lucida* Roxb.—Subarboreous shrubs, leaves opposite, decussate, rarely in whorls of three, stipules connate (annular), 'within the leaves', broadly ovate. All the leaves on a branch do not bear secondary branches in their axils. One to three branchless nodes intervene between two nodes bearing them (Fig. 1).

The node is trilacunar, the laterals are widely separated from the median. The *foliar foundations**** of the pair of opposite leaves at a node extend round the axis, their extending arms meet and coalesce to form a *collar**** confluent with the axis (Fig. 14).

The leaf-trace bundles move into the collar. The laterals branch, branches divert into the interpetiolar regions of the collar. On their way to the median

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*Names of species studied are given under their description.

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***For the origin and description of the *foliar foundation* see Majumdar, 1942; and of *collar*, White, 1955, and Majumdar and Pal, 1958*a*.

the laterals send out a branch each to the adaxial face of the petiolar region. All the secondary bundles divide and spread uniformly round the axis and send out another series of bundles immediately below the adaxial epidermis. This vascular adjustment takes place in the collar after it has separated from the axis (Fig. 15).

Separation of the collar from the axis begins in its interpetiolar regions (Fig. 14). After separation the collar grows upwards in the form of a tubular sheath with the petiolar regions intact. During further development the petiolar region separates from the collar to give rise to the petiole with three bundles. Its separation begins from outside, passes between the laterals and their branches and leaves a strip of tissue with the outer series of secondary bundles continuous with the interpetiolar portions of the collar. The collar thus separated from the axis and the petiole grows into a connate stipule with only the hypodermal series of bundles as its trace (Fig. 16). The developing bud bursts through the connate stipule.

Near the apex of the terminal bud a few meristematic cells differentiate in the collar opposite the median and form the primordium of the *foliar bud*. They are seen at the second node from the apex and are soon lost. The branch buds are *axial* in origin and get their vascular supply from the axial stele. They develop into branches* (Figs. 14, 49, 50).

2.   *Gardenia thunbergia* L.—Shrubs, leaves are in whorls of three, stipules large and connate (Fig. 2). In this species four to five branchless nodes come between two branch-bearing nodes.

The node is trilacunar as in the other species. The leaf-trace bundles are quite close to one another, and the subsequent behaviour of the laterals is similar to that in *G. lucida* (Fig. 17). The petiole separates with three bundles, and the collar develops into a connate stipule as in the other species.

The axillary bud is both *foliar* (Fig. 51) and *axial* in origin. In the shoot apex the buds remain foliar up to the next season, i.e., during the resting period, and when the buds unfold the branch buds develop in the lower nodes of the parent bud.

3.   *Portlandia grandiflora* L.—Shrubs, leaves opposite, stipules connate at base, broad, rounded at apex, splits open into two interpetiolar stipules in the upper region (Fig. 3). In this species the primary branches, which are more or less vertical, have secondary branches at each node, but the secondary branches behave differently : they bear 2-5 branchless nodes between two nodes with branches.

The node is trilacunar, the trace bundles are widely separated from one another. They move into the collar, and their behaviour in it is similar to that in the above species. The collar separates from the axis with the branches of the laterals and the petiole from the collar with the three leaf-trace bundles (Fig. 19). The collar now grows upwards in the form of a tubular sheath (Fig. 20). Its apical region separates into a pair of interpetiolar stipules (Fig. 21).

The bud primordia are both *axial* and *foliar* in origin. The foliar ones are ephemeral and leave socket-like depressions in the adaxial surface of the collar to indicate the position they occupied before falling off. The axial bud which develops into a branch separates from the collar and the axis by the formation of a *separation zone* developed completely surrounding it (Figs. 19, 52, 53).

4.   *Randia dumetorum* Lamk.—Shrubs or trees, leaves opposite, stipules ovate, acuminate, 'within the leaves', large, tapering and caducous. In this species the primary axial buds develop into thorns, and the branch buds originate secondarily on the axis between the thorn and the leaf at lower nodes (Fig. 4).

*Glands are a characteristics feature of the Rubiaceae. They are formed on the adaxial surface of the collar, particularly confined to the stipular region, and are of the nature of emergences (Mitra, 1948; Metcalfe and Chalk, 1950; Majumdar and Pal, 1958a). As they occur in all the species described they will be omitted from the description.
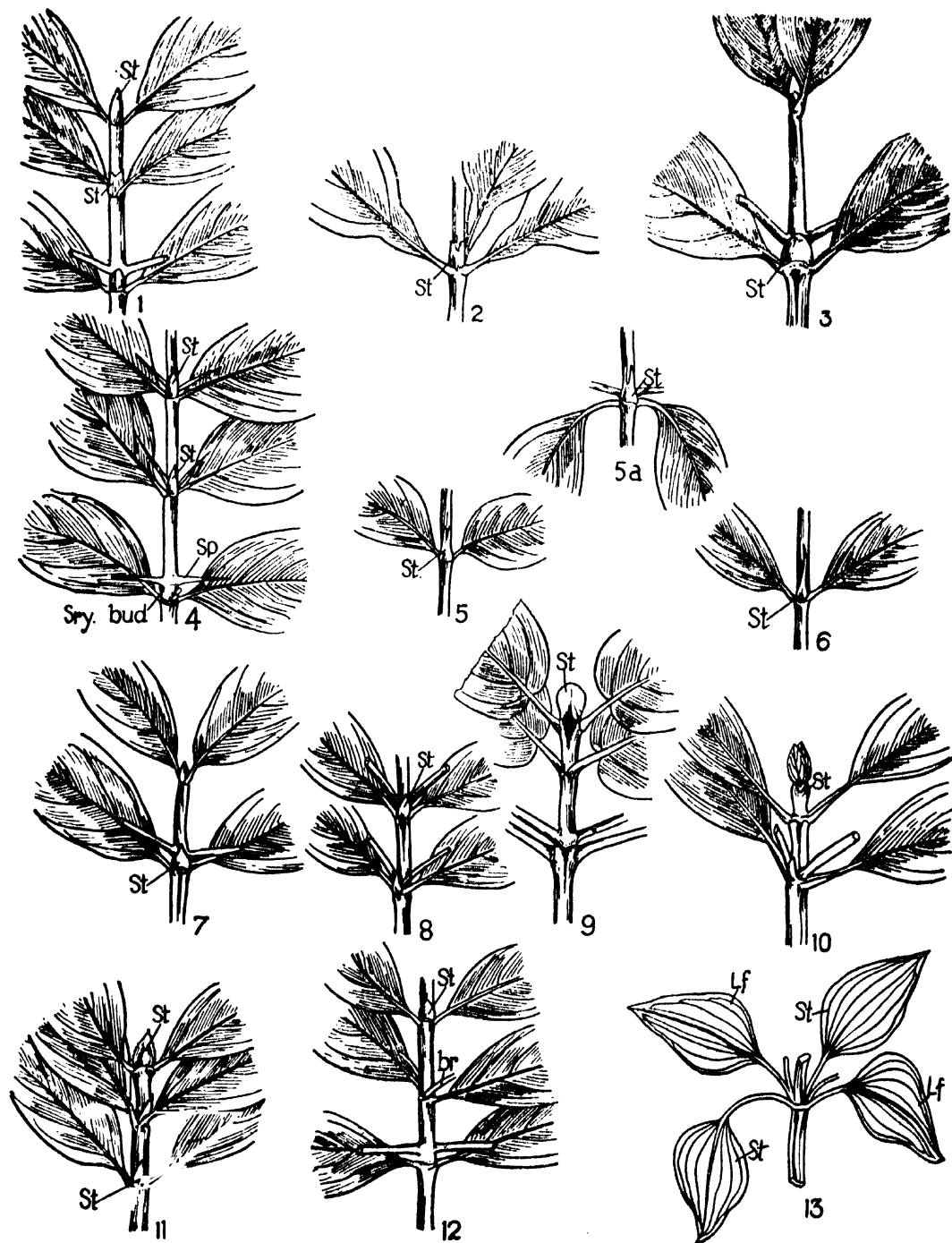
TEXT-FIGS. 1-13.

Figs. 1-13. represent respectively a portion of the adult shoot of each of the following thirteen species described in the text : *Gardenia lucida, G. thunbergia, Portlandia grandiflora, Randia dumetorum, Mussaenda frondosa, Luculia gratissima, Coffea arabica, C. bengalensis, Adina cordifolia, Morinda citrifolia, Hymenodictyon excelsum, Hamiltonia suaveolens,* and *Rubia edgeworthii.* They have been drawn from live specimens to show the nature of the stipules and branchings in these species.

The node is unilacunar, but the three leaf-trace bundles are discrete and depart for the collar together. The collar now separates from the axis and grows upwards in the form of a sheath. The laterals diverge into the collar where they divide and give rise to eight bundles on each side, four from each lateral. Two contiguous bundles from the opposite laterals unite to form the midrib bundle (composite) of each of the two stipules, into which the sheath spilts up soon after. The stipules overlap by their free margins at the upper region. In this species the petiole gets only the median as its bundle (Figs. 22, 23).

The axillary buds are both *foliar* and *axial* in origin (Fig. 54). The foliar buds are ephemeral, and the axial buds develop into thorns (cf. *Cateshea spinosa*). The branch buds originate later in the adult region between the thorn and the axillant leaf (Fig. 4).

5. *Mussaenda frondosa* L.—Shruby, leaves opposite, stipules long or short bi-fid, no connate growth at base (Fig. 5). Branching is very interesting in this species. In a twig the fourth and fifth nodes bear branches (decussate), sixth, seventh and eighth do not bear them, ninth and tenth nodes bear branches again, but the next five nodes are branchless (Fig. 5a). In another twig it was found that branches do not develop up to the tenth node, sometimes in place of a pair of branches at a node, only one developed (cf. *Galium mollugo*).

The node is unilacunar, but the leaf-trace consists of three juxtaposed bundles They move into the collar without severing connection with the central cylinder. They cause a wide gap in the axial cylinder when they sever their connection with it. The laterals diverge into the collar and divide to form the stipular bundles. The petiole receives only the median bundle (Figs. 24, 25). Separation of the collar from the axis starts simultaneously laterally and in front of the median external to the bud primordium.

The collar after its separation, from the axis and the petiole, breaks up into two interpetiolar stipules with the branches of the laterals. Number of bundles received by each stipule is ten to twelve, 5-6 from each lateral. The contiguous bundles do not unite but remain separate and the stipule becomes bi-fid.

In this species the node becomes laterally extended and downwardly projected for about 40 μ below the level of the node (Fig. 24). After the separation from the collar the horizontally extended portion of the axis forms a ledge or buttress upon which the next pair of foliar primordia are erected (Fig. 25).

Axillary buds are both *foliar* and *axial* in origin (Fig. 55). Foliar buds are ephemeral.

6. *Luculia gratissima* Sweet—Shrubs, leaves opposite, stipules cuspidate, deciduous. 4-5 branchless nodes come between two nodes with branches (Fig. 6).

The node is unilacunar. The single leaf-trace bundle moves into the collar. For a long time it remains unbranched and the collar grows confluent with the axis. Two separation zones organize in the interpetiolar regions of the collar, one on each side. These extend, meet and completely surround the axis. Before the collar separates from the axis the interpetiolar regions outgrow with a tendency to cover the petiolar regions (cf. *Paederia foetida*, Mitra, 1948), but these ear-like outgrowths are soon cast off by the sheathing collar (Fig. 26).

The petiole separates from the collar with only the central part of the trace bundle, but before the separation it gives out two branches to the collar from its free ends which give rise to the stipular bundles (Fig. 27). Each stipule thus gets two bundles, one from each leaf-trace bundle. The two bundles ultimately unite to form the only bundle (composite) of each stipule. The sheathing stipule then splits up into a pair of interpetiolar stipules in the upper region (Figs. 27, 28).

The *foliar* bud primordium is organized in front of the trace bundle but it soon disappears (Figs. 27, 56). The *axial* bud primordium which gets its vascular supply from the central cylinder, is surrounded by its *own separation zone* before the collar

is separated from the axis (Fig. 57). These branch-buds differentiate in the fourth node down below the axis, whereas the first foliar bud is seen to organize in the third node.

7. *Coffea arabica* L.—Shrubs, leaves opposite, stipules broad (Fig. 7). On a secondary branch 9-10 branchless nodes intervene between two nodes with branches.

The node is unilacunar. The leaf-trace bundle moves into the collar; its two free ends extend and soon branch off from its central part (Fig. 29). These branch bundles divide unequally, the smaller ones remain with the central part and the bigger ones spread in the collar. Each petiole thus gets 3 bundles—the central part of the leaf-trace bundle and two secondary bundles. The interpetiolar portions of the collar gets 9 bundles each, of which the central one is composite (Fig. 30). They overlap by their free margins in the upper region.

The axillary buds are both *foliar* and *axial* in origin (Figs. 58-60). Vascular supply to the foliar bud is rather peculiar in this species : the axial bud gets its supply from the central cylinder, and the foliar buds from the axial bud trace before the collar separates from the axis. This species differs from others in another respect : at the same node of the bud in the axil of one leaf of the pair, both the axillary buds are foliar; and in the axil of the opposite leaf both are axial (Figs. 61-65). In one bud at the same node foliar bud was found to develop 220 μ higher up on the collar whereas the axial one was at the node.

8. *Coffea bengalensis* Roxb.—Trees or shrubs, leaves opposite, stipules subulate. On branches which are more or less horizontal every leaf bears a branch, but on these secondary branches 3-13 nodes without branches intervene between two nodes with branches (Fig. 8).

The node is unilacunar; behaviour of the trace bundle is similar to that in the other species. The petiole gets three bundles, and the stipule two bundles, one each from opposite trace bundles which ultimately unite with each other to form a single composite bundle (Figs. 31-33). Towards the upper region the pair of stipules overlap by their free margins (cf. bud scales). In this species unlike in others the separation of the collar from the axis begins opposite the petiolar regions (Fig. 69).

Foliar buds get their vascular supply from the axial cylinder, but they do not develop into branches (Fig. 67). Foliar bud is initiated at the second node from the apex and the *axial* bud in older nodes. But in one case both foliar and axial buds were seen at the same node in the axil of the same leaf (Figs. 68, 69),

9. *Adina cordifolia* Benth. and Hook.—Trees, leaves opposite, deciduous, stipules orbicular, or oblong. 2-6 nodes without branches intervene between 2 nodes with branches (Fig. 9).

The central cylinder is somewhat wavy, oblong, with four sharp ridges and corresponding furrows two of which are shallow (Fig. 71). The node is unilacunar, each leaf-trace bundle is constituted of an entire furrow with the ridges flanking it. The later behaviour shows that the furrowed portion represents the median and the short ridges, the laterals. The three never sever their connection with one another (Figs. 34, 35). The laterals extend into the interpetiolar regions of the collar, divide and the branch bundles behave as in previous species. The petiole gets three bundles, and the interpetiolar regions eleven bundles each, the middle one being a composite one (Figs. 36, 37).

In this species both the petiole and the stipule show downward projections just free from the axis (Figs. 70, 71). These projections are noticed even when they are examined with a hand lens.

The bud primordium is *foliar* in origin and gets its vascular supply directly from the axial cylinder. The foliar buds do not develop and branches are developed from *axial* buds taking their origin at the lower nodes (Fig. 72).
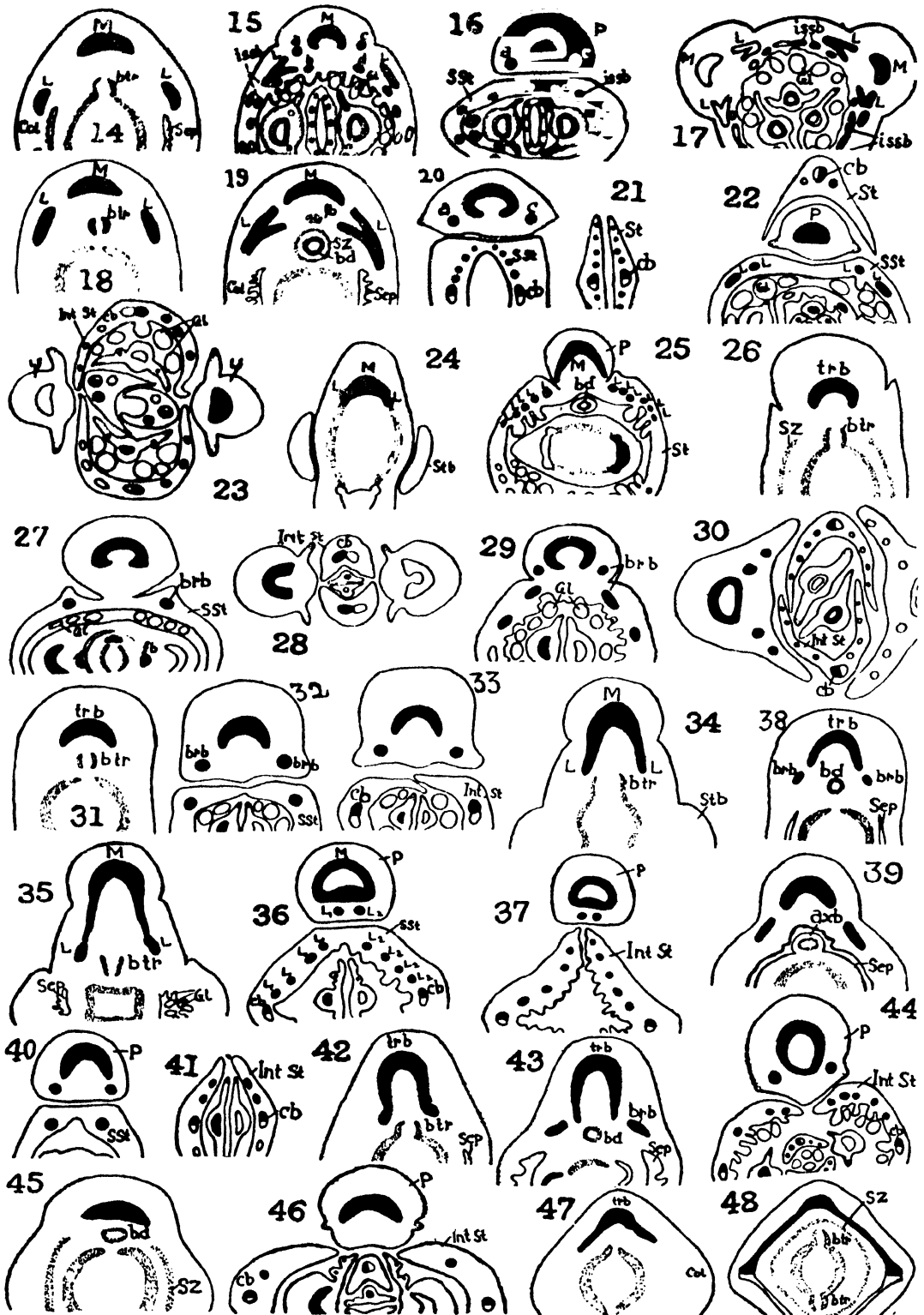
10. *Morinda citrifolia* L.—An elegant small tree, leaves opposite, short petioled, stipules large, broadly oblong, semilunar, entire, smooth or two fid. Normally one to three branchless nodes intervene between two nodes with branches. But in some branches all the nodes were found to bear lateral branches (Fig. 10).

The node is unilacunar, the single bundle of the leaf-trace moves into the collar from where it sends out branches into its interpetiolar regions (Fig. 38). The petiole receives three bundles. The collar separates from the axis and the petiole from the collar in the usual way. The stipule grows at first as a sheath and then splits up into a pair of interpetiolar stipules at the upper region. In the lower region each stipule gets five bundles with the central one composite, but their number is gradually reduced to three only, the composite midrib and two laterals (Fig. 40, 41).

Foliar buds originate at the third node and the *axial* at the fourth from the tip. The foliar bud gets its vascular supply from the central cylinder before the collar separates from the axis, but they do not develop into branches. Branch buds are always *axial* in origin (Figs. 39, 73).

11. *Hymenodictyon excelsum* Wall—Trees or shrubs, leaves opposite, stipules subentire. Seven to sixteen branchless nodes intervene between two nodes with branches (Fig. 11).

The node is unilacunar; the single trace bundle moves into the collar and its subsequent behaviour is similar to that of the previous species. The petiole separates with three bundles and the stipules get 7-9 bundles each, of which the central one is composite (Figs. 42-44).

Buds are both *foliar* (Fig. 74) and *axial* (Fig. 75) in origin. The foliar is ephemeral, the axial develops into a branch with vascular supply from the central cylinder.

12. *Hamiltonia suaveolens* Roxb.—An undershrub with spreading branches, leaves opposite, stipules short, acute. Prain describes the stipule as intrapetiolar and persistent. Two to ten branchless nodes intervene between two nodes with branches (Fig. 12).

The node is unilacunar. Trace bundle moves into the collar where its behaviour is similar to that in other species with one-bundle leaf-trace. A distinct but faint separation zone is formed between the axis and the collar. The petiole receives only the central part of the trace bundle and the interpetiolar regions of the collar four bundles on each side, but the interpetiolar stipules get three bundles each, the central one being composite (Figs. 45, 46).

EXPLANATION OF FIGURES

Figs. 14–48.—are camera lucida drawings, magnification of each figure has been noted against it. The figures are mostly from transverse sections (t.s.) of the shoot apices, and show the collar, the mode of its separation from the axis, the origin of sheathing and interpetiolar stipules, their vascularization, etc., and the glands.

Figs. 14–16.—*Gardenia lucida*, t.s. ×50.
Fig. 17.   —*Gardenia thunbergia*, t.s. ×50.
Figs. 18–21.—*Portlandia grandiflora*, t.s. ×50. Fig. 19 also shows the axial bud, and its separation zone (*sz*).
Figs. 22–23.—*Randia dumetorum*, t.s. ×75.
Figs. 24–25.—*Mussaenda frondosa*, t.s. ×50.
Figs. 26–28.—*Luculia gratissima*, t.s. ×50.
Figs. 29–30.—*Coffea arabica*, t.s. ×100.
Figs. 31–33.—*Coffea bengalensis*, t.s. ×100.
Figs. 34–37.—*Adina cordifolia*, t.s. ×50.
Figs. 38–41.—*Morinda citrifolia*, t.s. ×30. Fig. 39 also shows axial bud.
Figs. 42–44.—*Hymenodictyon excelsum*, t.s. ×30.
Figs. 45–46.—*Hamiltonia suaveolens*, t.s. ×30.
Figs. 47–48.—*Rubia edgeworthii*, t.s. ×100.

The bud primordium is *foliar* in origin (Fig. 76). It receives its vascular supply from the axial cylinder before the collar separates from the axis. It is not always ephemeral. *Axial* buds originate from the lower nodes (Fig. 77).

13. *Rubia edgeworthii* Hook.—Herbs, scandent, leaves opposite, stipules stalked, foliaceous, morphologically similar to leaves in size, shape and vascularization. Branching regular (Fig. 13).

The node is unilacunar. The leaf-trace-bundle moves into the collar, but its behaviour is quite different from that in the species described with one-bundle leaf-trace. The trace bundles of the opposite pair of leaves extend by their arms which meet and fuse to form a complete girdle round the axis (cf. *Galium mollugo, Rubia cordifolia*). From the region of their union a branch (jointly contributed) is sent towards the periphery of the collar which enters the stipule as its trace (Figs. 47, 48).

A feeble separation zone is seen to differentiate at the junction of the collar and the axis, but actual separation starts external to the bud primordium, which is thus axial in origin and gets its vascular supply directly from the axial cylinder. *No foliar bud* has been seen to organize in this species (Fig. 78).

<div align="center">DISCUSSION</div>

I. The species studied show the following external features :
1. In habit they range from trees to scandent herbs.
2. Branching is noteworthy. All the leaves on the stem or parent axis do not bear secondary branches. Between two successive branch-bearing nodes come nodes, one to sixteen, without branches.
3. Leaves are petiolate, occur in pairs except in *Gardenia thunbergia* where they are normally in whorls of three, and in *G. lucida* which occasionally shows a node with three leaves.
4. All the leaves are stipulate. The stipules are *interpetiolar, sheathing* (connate), *partly sheathing* and *partly interpetiolar* and *foliaceous* (see under Stipules).
5. The leaves are all *complete*, i.e., each leaf consists of three parts : the *blade*, the *petiole* and the *leaf-base*.

II. *The Leaf-base :*
1. The foliar foundations unite by their extending arms around the axis and form a collar confluent with it. In all the species reported here the collar grows upwards, free from the axis, in the form of a tubular sheath. All vascular adjustments for the petiole and stipules take place in the collar. (Mitra and Majumdar 1952, Majumdar, 1955a, 1956; Majumdar and Pal, 1958a).

The foliar foundation represents the base of the leaf (leaf-base). This has been established on the evidence of its initiation, ontogeny, anatomy and vascularization at the shoot apex (Majumdar, 1955a, 1956; Mitra, 1949). Support for this finding comes very recently from White (1955) who states that leaf-bases of each pair (of decussate leaves) of *Acer pseudoplatanus* are extended laterally and fused to form a collar (or collet) surrounding the axis (P. 437). Long before Goebel (1889) and Ganong (1894) thought that only the leaf-base surrounded the stem (Boke, 1955, p. 1). Boke supports Ganong's conclusion that 'the stem is surrounded by decurrent leaf-bases' which in its turn supports the leaf-skin theory of Saunders (1922) (cf. berindung theory of Hofmeister, 1851, and mantle-core theory of Mitra and Majumdar, 1952, for the constitution of the internode). The collar in the Rubiaceae, therefore, represents the leaf-bases united by their outer margins. It consists of two parts : the lower confluent with the axis, and the upper free from it. When the free portion of the collar is absent, as in China-rose, Jasminum, etc.,

and its lower portion entirely incorporated in the axis as its outer mantle, the leaf is held without a base (as done by the American botanists, see Majumdar, 1955a) and the stipules, if present, are regarded as *cauline* (Parkin, 1948).

Strasburger (1930) stated that the petiole is never inserted directly on the axis. It appears to be correct only when the foliar foundation or the collar is taken to represent the leaf-base. In many cases the sheathing base of the leaf is described as the base of the petiole flattened or expanded. But this is not supported by the phylogeny and ontogeny of this organ. The petiole is a later addition to the leaf, and is intercalated between the blade and the leaf-base (Majumdar, 1957).

2. Each collar, confluent or free, has two regions : the *petiolar* and the *inter-petiolar*. The petiolar region is radially extended and contains only the median, or the median and the laterals of a three-bundle leaf-trace, or in the case of a single-bundle leaf-trace, its central part with or without two other secondary bundles given out by the extended free ends of this bundle.

3. The petiolar region separates from the rest of the collar and grows into the petiole with three or one bundle as the case may be. The petiolar region, the petiole and the midrib of the blade are continuous, and the sheathing base, as we have mentioned before, is often mistaken for the petiole with expanded base (e.g. *Panax*, *Heracleum*, etc.).

4. After separation of the petioles (leaves opposite) the collar may immediately resolve into two interpetiolar stipules, or may grow up for some length before being split up into a pair of interpetiolar stipules, or may grow up and completely enclose the bud as a connate stipule, or may give rise to foliaceous stipules as lateral append-ages like the leaves (see under Stipules).

III. *The Stipules :* Stipules are outgrowths of the leaf-base at and above the level of separation of the petiole from the former. In twelve species they are sessile when they may be regarded as the continued upgrowth of the collar, and in the thirteenth species, i.e., in *Rubia edgeworthii*, the stipules are stalked and lateral in origin like the leaves.

The stipules in all the thirteen species can be divided into the following types :
1. *Interpetiolar*—a pair of stipules, one from each leaf, on the same side of the axis, united by their outer margins. They may be :
    (i) *Triangular*—broad and/or acuminate or acute with receding margins towards the apex. There is no connate growth at the base, and the collar splits up and grow into a pair of stipules immediately after the separation of the petioles, e.g. *Mussaenda*, *Hymenodictyon* and *Hamiltonia*.
    (ii) *Connate at base*, split open into a pair of interpetiolar stipules near the upper region :
        (a) connate for a very short distance, about $20-25\mu$, e.g., *Coffea* spp., *Adina*, and *Morinda;*
        (b) connate for about 500μ above shoot apex—e.g. *Randia;*
        (c) connate for about $1000—1500\mu$ above shoot apex—e.g. *Portlandia*, *Luculia;* or
        (d) overlapping by their inner margins near the tip : *Coffea arabica*—800μ, *Coffea bengalensis*—520μ, and *Randia dumetorum*—160μ above the shoot tip which they completely enclose.
2. *Sheathing :*—*Connate all throughout.* All the four (leaves opposite), or six (leaves in whorls of three) stipules are united by both the margins up to the tip. They form only one stipule which has been described as *connate*, e.g. *Gardenia lucida*, *G. thunbergia.*

Willis (1951) writes : the stipules in the Rubiaceae stand between the petioles (interpetiolar), or between the petioles and the axis (intrapetiolar or axillary), and are frequently united to one another and to the petiole, so that a sheath is formed round the stem (pp. 573-574).

But true intrapetiolar (axillary) stipules we have not found in the twenty-four species of the Rubiaceae so far studied by us. The sheath is not formed in the way suggested by Willis. In the species described the *sheath* and the *sheathing stipules* are formed in the following ways :

    (i) *Sheath :* The collar after being free from the axis grows upwards in the form of a tubular sheath with the petiolar regions. The sheath is formed by the union of the leaf-bases, and the petiolar regions represent the foliar foundations (*Soubassement foliares* of Gregoire, 1935) at their initiation at the shoot apex.

    (ii) *Sheathing stipule*—In the case of connate stipules the interpetiolar regions of the collar with a strip of the adaxial tissue of the petiolar regions separate from the petiolar regions (now petioles) and grows upwards in the form of a *sheathing stipule*, as in the *Gardenia* spp. In the case where the stipule is sheathing at the base but split open at the upper region the sheathing portion is formed in the way described above.

Willis made the evident mistake by confusing the petiolar region of the collar with the petiole proper. The nature of the foliar foundation as the base of leaves came to be realised only lately.

Whether the collar (united leaf-bases) would outgrow into a pair of interpetiolar stipules or a sheathing stipule after separation from the petioles depends on the disposition of branch bundles (stipular traces) in the adaxial face of the collar (see below).

3. *Foliaceous :* Foliaceous stipules are leaf-like in shape, size and vascularization and are stalked like the leaves of whose bases they are outgrowths, e.g. *Rubia* spp. The three genera, *Galium, Rubia* and *Asperula* 'are characterised by the resemblance of the stipules to the leaves'.

IV. *Vascular Supply to the Stipules :* Vascular supply to the leaf-base of which the stipules are outgrowths, may be described according to the number of bundles which constitute each leaf-trace. Thus :

    (i) *Node trilacunar*—trace bundles three, widely separated from one another, e.g. *Gardenia lucida* and *Portlandia*, but they are closer in *G. thunbergia*.

    (ii) *Node unilacunar*— trace bundles three, but they depart for the collar together leaving a wide gap in the axial stele, e.g. *Mussaenda, Randia*.

    (iii) *Node unilacunar*—trace bundle consists of three juxtaposed bundles, e.g. *Adina*.

    (iv) *Node unilacunar*—trace bundle one, e.g. *Luculia, Hymenodictyon, Hamiltonia, Coffea, Morinda* and *Rubia*.

From a close study of the distribution of trace bundles in the above species it would appear that there may be some truth in the statement of Sinnott and Bailey (1914) that the unilacunar condition of the node arose either by the approximation of the three trace bundles to one another, or by *complete merger* of the laterals with the median. Suppression of the laterals to give rise to the unilacunar condition has not been observed in any of the species studied.

In the final disposition of the vascular bundles from the axial stele or cylinder the petiole in the majority of cases gets three bundles of which the central one is the median or its equivalent, and the stipule gets the laterals and/or their branches. The distribution is noted below :

Gardenia—in both the species the laterals send out branches (by division) to the interpetiolar regions of the collar and also to the adaxial side of its petiolar region. These branch bundles in their turn send branches to form a second series of bundles to run parallel to the inner face of the collar. The stipule (connate), however, gets only the second series of bundles, equally spaced all round, as its vascular system.

Portlandia—Each stipule gets 9 branch bundles of which the central one is a composite one. The branch bundles are not equally spaced, but leave a wide gap opposite the median. The stipule which is connate at the base splits open in this gap due perhaps to vigorous growth upwards round the midrib bundle.

Mussaenda—Each stipule gets 10 or 12 branch bundles the central ones do not unite but grow independently and a bi-fid stipule is formed.

Each stipule in *Randia* gets 7, in *Coffea arabica* 9, *Coffea bengalensis*–1, *Luculia*–1, *Adina*–11, *Morinda*–5, *Hymenodictyon*–7, *Hamiltonia*–3, and *Rubia*–1—the central bundle in all cases is a composite (contributed by two opposite laterals) one.

The stipules in twelve species are the results of vertical up-growth of the united leaf-bases with branches of the laterals or their equivalents as their vascular supply. In *Rubia*, however, the stipule is a stalked lateral outgrowth with a trace which is sent towards the periphery jointly by both the leaf-traces. The origin and development of the sheathing stipules can be compared with those (ochrea) of *Polygonum orientale* (Mitra, 1945; Majumdar, 1956).

V. *Methods of separation of the collar from the axis*.—In every species studied the collar separates from the axis and grows upwards in the form of a tubular sheath. In *Rubia* a distinct circular separation zone is formed at the junction of the collar and the axis. This zone also gives rise to glands (cf. *Galium*). In *Luculia* and *Hamiltonia* the origin of the separation zone is confined to the lateral regions. In the majority of cases the actual separation starts from the lateral regions but in *Coffea bengalensis* and *Rubia* the separation begins from opposite the median bundle.

In the majority of cases the separation of the leaf-base from the axis appears to be due to the unequal vertical growth of the axis and the collar. This fact also leads to the suggestion that the axis and the collar belong to different organizations. Kundu and Rao (1957) noticed in *Boehmeria* "differences in growth rates of the central and peripheral zones of the axis", the peripheral zone might mean the *collar*.

VI. *Separation of the petiole from the collar*.—After it has separated from the axis the collar grows upwards in the form of a connate sheath for some time before its petiolar regions separate to grow into the petioles. It separates from the collar in two ways :

  (i) In *Mussaenda, Hymenodictyon* and *Hamiltonia* where the connate leaf-base after the separation of the petiolar regions directly grows into a pair of interpetiolar stipules, the separation starting from outside proceeds obliquely towards the centre of the petiolar region on the adaxial side and opens into the cavity just in front of the median bundle. The petiole assumes a biconvex shape, the inner more projecting and sharp than the outer one (Figs. 25, 44, 46).

  (ii) In all the other species where the base of the stipule is connate, but splits up into two interpetiolar stipules at the upper region, the separation starts from outside and proceeds inwards between the laterals and their branches almost horizontally or slightly convexly leaving a strip of tissue with the branch bundles continuous with the interpetiolar regions of the collar (Figs. 21, 23, 28, 30, 33, 37, 41).

In the case of *Gardenia* stipular bundles are uniformly distributed in its tissue around the axis, and the upward growth of the stipule is without any break, but in the other species the bundles leave wide gap in front of the median, and the splitting takes place in this gap. In *Rubia* the origin and development of the stipule is like that in *Galium mollugo* (Majumdar and Pal, 1958a) and in *Rubia cordifolia* (Majumdar and Pal, 1958b).

THE AXILLARY BUD

The axillary bud and its axillant leaf are closely associated together. These buds are important in the life of a plant as their presence or absence gives the latter its habit and form.

At least three problems relating to the axillary bud still remain unsolved and they are : its origin, vascularization and its morphology and relationship with its axillant leaf.

In the majority of cases so far investigated the origin of the axillary bud has been reported as *axial* from the 'detached meristem'** (Koch, 1893; Goebel, 1905, Louis, 1935; Reeve, 1943; Sifton, 1944; Miller and Wetmore, 1946; Garrison, 1949*a*, *b*; 1955; Philipson, 1949; Sterling, 1949; Gifford, 1951; Kundu and Rao, 1954, 1955, amongst others). *Foliar* origin has been reported only in a very few cases (Vöchting, 1873-74; Leinfellner, 1937; Majumdar, 1942; Majumdar and Datta, 1946). In monocotyledons, at least in the Gramineae, the axillary bud is axial in origin (Sharman, 1942, 1945; Hsü, 1944; Ledin, 1954; Saha, 1954).

Bud traces are mostly *acropetal* in differentiation from the central cylinder. In the majority of cases two bundles leave for the bud from the ends of the central cylinder flanking the gap caused in it by the departure of the median strand of the axillant leaf. Their *basipetal* differentiation has been reported in the case of foliar buds.

Garrison (1955) considers the above as 'contrasting viewpoints' (p. 263). I do not know if she meant that there should be only one type of origin and one type of vascularization for all axillary buds.

It is now agreed that axillary buds may originate close to the apical meristem of the parent shoot, or at some distance from the apical meristem. Esau (1953) relates this difference in origin to the time and position of bud development. Philipson (1949) has suggested that the bud-trace differentiation is related to time of bud development. He writes : In buds that develop simultaneously with the leaves (near the apex) the vascular strands arise acropetally, whereas traces of buds which develop long after the leaf, arise basipetally because they become seats of renewed meristematic activity located in vacuolated tissue.

Kundu and Rao (1955, 1957) have in their later contributions on the subject suggested that the origin of buds might be axillary, foliar and cauline, and each of them after their origin may assume any of the other positions by adjustment during growth and development. But the authors do not explain what they meant by cauline and axillary origin of buds. Esau points out that axillary buds generally arise on the stem, and therefore cauline in origin. (We are not dealing with extra-axillary, supra-axillary and accessory buds which are always cauline in origin). She thinks the term axillary is, therefore, somewhat inaccurate. But I think, at least for descriptive purposes, it is quite accurate if the bud is found in the axil of the axillant leaf.

But what is meant by *axil*? Axil (L. *axilla*—armpit) is the upper angle formed by a leaf and the supporting stem (Heinig, 1899), syn. intrafoliaceous. But at its insertion on the axis the free limb of a leaf embraces quite a portion of the axis. The axil, therefore, should be, as I think, the angle of contact between the leaf opposite its median bundle and the axis. Only the buds which occupy this angle are to be called axillary, whether the origin be foliar or axial, and other buds lateral to it should be regarded as accessory. Thus :

**‘A group of cells which at one time formed part of the apical meristem and which have retained their meristematic potentialities’ (Wardlaw, 1952).

Axillary bud   Axial (cauline)
(positional)   Foliar
                 At the time of origin neither axial nor foliar.

The relationship with the axillant leaf is not clear. Warming (1872) suggested that the axillant leaf and its bud constitute one unit. According to him the axillant leaf might be treated as the first and only leaf of the axillary bud (see for further and fuller account, Arber, 1950; Majumdar, 1955b). No one seems to have pursued the theme further.

With regard to its morphological nature Arber (1950) offered a theory : According to her the axillary bud is the offspring of the leaf, the partial shoot, the product of its basal lobes (p. 126). The primitive leaf was taken to be a trilobed one. She elaborated the arguments on which she based her theory. Majumdar (1955b) suggested a theory alternative to that of Arber. According to him the axillant leaf and the axillary bud represent two primordia of the distal forking of a branch. The outer one (positional) develops quickly and rapidly to form the leaf primordium under the influence of its acropetally differentiating median strand which follows its erection closely. The other (inner) remains undeveloped until its trace comes from the axial cylinder a little later than the median trace bundle of the leaf' (1955b, pp. 91-92). Thus according to Majumdar the axillant leaf and its axillary bud represent a twin structure instead of the parent and its offspring. The position of the bud in relation to its primordium also appears to lend support to the theory of Majumdar.

The Rubiaceae appear to be unique in so far that the origin of the axillary bud is definitely both *foliar* and *axial* in origin, and they occur in the same bud, and sometimes at the same node. The foliar buds are mostly ephemeral, and branches develop from the axial buds which always receive their vascular supply from the central stele. At least in half a dozen species, in the axils of the same leaf, twin foliar buds, twin axial buds and both foliar and axial buds occur at the same node. Another noted fact is that bud initials arise by de-differentiation of vacuolating dividing cells of the leaves (foliar) and axis (axial).

The following account gives a summary of observations of 24* species so far studied :

I. Origin of the bud : Either axial or foliar, but not both in the same bud

Species	Origin	Vascularization	Development
1. *Rubia edgeworthii* (Fig. 78)	Axial	Acropetal	into a branch
2. *R. cordifolia* (Fig. 79) (Majumdar and Pal, 1958b)	Axial	Acropetal	,, branch
3. *Catesbea spinosa* (Figs. 80-82) (Majumdar and Pal, 1958b)	Axial	Acropetal	,, spine; branch-buds arise secondarily between the spines and the leaf at node 6 below (cf. Randia)
4. *Anthocephalus cadamba* (Fig. 83) (Majumdar and Pal, 1959)	Foliar	Acropetal	into a branch
5. *Cinchona ledgeriana* (Figs. 84,85) (Majumdar and Pal, 1958b)	Foliar	Acropetal	,, branch

*Thirteen species have been described in this paper, the rest are from papers already published (Majumdar and Pal, 1958a, 1958b).

In all the five species the bud primordium differentiates in the ground tissue at the junction of the axis and the collar. Separation of the collar from the axis starts in the interpetiolar regions and proceeds towards the median bundles of the leaf-traces. If the line of separation passes external to the bud primordium it becomes *axial*, if internal to it the bud primordium goes with the collar and becomes *foliar*. In the earlier stages of bud initiation it can not, therefore, be strictly assigned either to leaf or to the axis.

II. ORIGIN OF THE BUD : AXIAL OR FOLIAR AT THE SAME OR DIFFERENT NODES OF THE SAME BUD

6. *Gardenia lucida* (Figs. 49-50)

At the same or different nodes :

Foliar—a few meristematic cells, no vascular supply, ephemeral, first appearance at second node from apex.

Axial—acropetal vas. supply, develops into branch, in lower nodes.

Branching—1-3 branchless nodes between 2 nodes with branches.

7. *G. thunbergia* (Fig. 51)

Foliar—acropetal, soon falls off, at upper nodes.

Axial—acropetal. develops into branch, at lower nodes.

Branching—4-5 branchless nodes intervene between 2 nodes with branches.

8. *G. florida* (Fig. 86, 87) (Majumdar and Pal, 1958*b*)

Foliar—at the upper node, ephemeral.

Axial—at lower nodes, acropetal vascular supply, develops into branch.

Branching—1-3 branchless nodes intervene between two nodes with branches.

9. *Portlandia grandiflora* (Fig. 52-53)

At different and same nodes :

Foliar—only a few meristematic cells, no vascular supply, ephemeral, upper node.

Axial—bud primordium originates at junction of collar and axis : it separates from both collar and axis by a separation zone of its own, vascular supply acropetal, develops into a branch. From the manner of origin and separation it can not properly be referred to either axis or leaf.

Branching—2-5 branchless nodes come between two nodes with branches.

10. *Randia dumetorum* (Fig. 54)

Same and different nodes :

Foliar—no vascular supply, ephemeral.

Axial—acropetal vas supply, develops into thorn.

Axial—secondary in origin between the thorn and the leaf in lower nodes which develops into branches.

11. *Mussaenda frondosa* (Fig. 55)

origin at different nodes:

Foliar—no vascular supply, ephemeral, arises at upper nodes.

Axial—acropetal, at lower nodes, develops into branches.

Branching—3-10 branchless nodes between 2 nodes with branches.

12. *Luculia gratissima* (Fig. 56, 57)

at different nodes:

Foliar—no vascular supply, ephemeral, at upper nodes.

Axial—acropetal vas. supply, the bud separates from the axis and the leaf by the formation of a separation zone around itself. Therefore, like that in *Portlandia* its origin is independent (?) of the leaf and the axis.

Branching—4-5 branchless nodes come between two nodes with branches.

13. *Coffea arabica* (Figs. 58-65): This species is unique among the species so far examined. It shows three types of axillary buds in the same bud :

(i) Foliar and foliar as twin buds in the axil of the same leaf of a node, the distal gets vascular supply from the axial cylinder, and the proximal from the distal (Figs. 61-63).

(ii) Axial and axial as twin buds in the axil of the opposite leaf of the above node; the proximal gets its vascular supply from the axial cylinder and the distal from the proximal bud, (Figs. 64, 65) and

(iii) Foliar and axial at a different node with vascular supply from the axial cylinder (Figs. 58-60).

14. *Coffea bengalensis* (Figs. 66-69)

Foliar—acropetal, at upper node.

Foliar and axial—at the same node, as *twin* in the axil of the same leaf; the distal gets its vascular supply from the axial cylinder and the foliar from the axial bud trace.

15. *Adina cordifolia* (Fig. 72)

at different nodes—

Foliar—acropetal vas. supply, falls off soon, at upper nodes.

Axial—acropetal, develops into a branch, at lower nodes.

Branching—2-6 branchless nodes between two nodes with branches.

16. *Morinda citrifolia* (Figs. 39, 73)

at different nodes :

Foliar—acropetal vas. supply, falls off soon, at upper nodes.

Axial—acropetal, develops into a branch, origin at lower nodes.

Branching—1-3 branchless nodes between 2 nodes with branches.

17. *Hymenodictyon excelsum* (Figs. 74, 75)

at different nodes :

Foliar—acropetal vas. supply, falls off soon, at upper nodes.

Axial—acropetal, develops into branch, at lower nodes.

Branching—7-16 branchless nodes between 2 nodes with branches.

18. *Hamiltonia suaveolens* (Figs. 76, 77)

at different nodes :

Foliar—acropetal vas. supply, falls off soon, at upper nodes.

Axial—acropetal, develops into branch, at lower nodes.

Branching—2-10 branchless nodes between 2 nodes with branches.

19. *Hamelia patens*
    (Figs. 88-91)
    (Majumdar and Pal, 1959)

at different nodes—Buds originate at junction of axis and collar, axial or foliar is determined by the line of separation passing external or internal to it (cf. spp. 1-5 above)

Foliar—acropetal vas. supply, falls off soon, first seen at node three from tip (Figs.   88-90).

Axial—acropetal, develops into branch, at 6th node below (Fig. 91).

Branching—2-4 branchless nodes between 2 nodes with branches.

20. *H. sphaerocarpa*
    (Figs. 92, 93)
    (Majumdar and Pal, 1959)

as in the other species.

Foliar—acroptel vas. supply. ephemeral, first noticed at node 2 (Fig. 92).

Axial—acropetal, develops into branch, at 6th node (Fig. 93).

21. *Galium mollugo*
    (Figs. 94. 95)
    (Majumdar and Pal, 1958*a*)

Three types of bud formation are noticed in this species :

    (i) Foliar—at the same node, one each in the axils of the pair of opposite leaves.

    (ii) Axial—at the same node, one each in the axil of the pair of opposite leaves (Fig. 94).

    (iii) Foliar and foliar as a *twin* at the same node in the axil of the same leaf (Fig. 95).

22. *Oldenlandia Heynii*
    (Figs. 96-98)
    (Majumdar and Pal, 1958*b*)

Foliar—Two foliar buds (*twin*) organize at the same node in the axil of the same leaf; the distal one gets its vascular supply from the axial cylinder, and the other represented by a few meristematic cells gets its very feeble vascular supply from the median trace bundle. The distal bud develops into a very small bud (Figs. 96, 97).

Axial—acropetal, develops into a branch, arises in the lower nodes (Fig. 98).

Branching—double branches are found in the axil of the same leaf, one remains small.

EXPLANATION OF PLATE XI.

(*Axillary Buds*)

Figs. 49–73.—are microphotographs of transverse (t.s.) and longitudinal (l.s.) sections of the apical bud to show particularly the origin, development and vascularization of the axillary buds, foliar and axial, For details see text.

Figs. 49–50.—*Gardenia lucida*, t.s.

Fig. 51.   —*Gardenia thunbergia*, t.s.

Figs. 52–53.—*Portlandia grandiflora*, t.s.

Fig. 54.   —*Randia dumetorum*, t.s.

Fig. 55.   —*Mussaenda frondosa*, t.s.

Figs. 56–57.—*Luculia gratissima*, t.s.

Figs. 58–65.—*Coffea arabica*, t.s.

Figs. 66–69.—*Coffea bengalensis*, t.s.

Figs. 70–72.—*Adina cordifolia*, t.s., but fig. 70 is a logisection to show the stipule bases free from axis.

Figs. 73.   —*Morinda citrifolia*, t.s.

PAL.

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23. *Dentella repens*
    (Figs. 99-102)
    (Majumdar and Pal, 1958*b*)

24. *Stephagyne parviflora*
    (Figs. 103-104)
    (Majumdar and Pal, 1958*b*)

Foliar—ephemeral at upper nodes (Fig. 102).

Branches—double are found at each node, one remains small. Axial and axial as a pair of *twin* and foliar buds originate at the same node, the proximal gets its vascular supply from the axial cylinder, and the distal from the bud trace. The foliar is represented only by a few meristematic cells (Figs. 99-101).

Foliar (?)—originates at the junction of the leaf and axis, vascular supply from the axial cylinder, a separation zone separates it from the axis, falls off soon (Fig. 103).

Axial and axial as twin buds arise at the same node in the axil of the same leaf, the distal degenerates and the proximal develops into a branch. The distal gets its vascular supply from the proximal, and both separate from the axis and the collar by their own separation zone formed round them (Fig. 104).

From the foregoing account of the origin and vascularization of the axillary buds in twenty-four species of the Rubiaceae the following conclusions are inevitable :

I. *Their origin :* Axillary buds may be *foliar* and/or *axial* in origin.

1. In some cases the foliar or axial origin of the bud is determined by the line separating the collar from the axis passing external (axial) or internal (foliar) to the bud which takes its origin in the ground tissue at the junction of the two organs. The buds receive their vascular supply from the axial cylinder as in *Rubia, Catesbea, Anthocephalus, Cinchona, Stephagyne, Hamelia, Gardenia, Mussaenda, Adina, Morinda, Hymenodictyon* and *Hamiltonia*.

2. In some cases the origin of the bud is similar to that in (1). but they separate from the axis and the collar by the formation of a separation zone round each of them as in *Portlandia* and *Luculia*.

N.B.—In (1) and (2) at the time of the origin a bud is neither axial nor foliar.

3. The foliar buds, which in most cases appear at upper nodes of the bud, are mostly ephemeral, with or without vascular supply, whereas the axial buds which appear at lower nodes of the same bud with vascular supply from the axial cylinder, develop into branches. This is reflected in the nature of branching on adult stems.

4. In some species, e.g. *Catesbea* and *Randia*, the branch buds arise secondarily on the axis between the spine or thorn and the leaf at the node.

EXPLANATION OF PLATE XII

(*Auxiliary Buds* continued)

Figs. 74–75.—*Hymenodictyon excelsum*, t.s.
Figs. 76–77.—*Hamiltonia suaveolens*, t.s.
Fig. 78.   —*Rubia edgeworthii*, t.s.
Fig. 79.   —*Rubia cordifolia*, t.s.
Figs. 80–82.—*Catesbea spinosa*. t.s.
Fig. 83.   —*Anthocephalus cadamba*, t.s.
Figs. 84–85.—*Cinchona ledgeriana*, t.s.
Figs. 86–87.—*Gardenia florida*, t.s.
Figs. 88–91.—*Hamelia patens*, t.s.
Figs. 92–93.—*Hamelia sphaerocarpa*, t.s.
Figs. 94–95.—*Galium mollugo*, t.s.
Figs. 96–98.—*Oldenlandia Heynii*, t.s.
Figs. 99–102.—*Dentella repens*, t.s.
Figs. 103–104.—*Stephagyne parviflora*, t.s.

3

5. In some species, e.g. the foliar and axial buds originate as twins at the same node and in the axil of the same leaf, e.g. *Randia*, *Coffea arabica*, *C. bengalensis* and *Portlandia*.

6. In some species, e.g. *Oldenlandia*, *Galium*, *Coffea arabica*, foliar and foliar buds originate as twins at the same node in the axil of the same leaf.

7. In some species, such as, *Dent lla*, *Stephagyne* and *C. arabica*, axial and axial buds as twins arise at the same node in the axil of the same leaf.

8. In *Dentella* there occur three buds, foliar, axial and axial in the axil of the same leaf.

II. *Their relationship with the axillant leaves, and morphological nature :*

It is indeed difficult to suggest any relationship between the axillant leaf and its bud. In a few species the bud at its initiation is not at all connected directly with the axis or the leaf. In the majority of cases the foliar buds take their origin in the tissues of the leaf, and the axial in the tissues of the axis. In one case a foliar bud has been seen to take origin 220μ above the shoot apex (e.g. *Coffea arabica*). The axillant leaf cannot, therefore, be regarded as the first leaf of the bud as suggested by Warming (1872).

Arber's (1950) parent-offspring theory is not supported. There is no indication that the bud is produced by the face to face union of the basal lobes of the axillant leaf. Our observations, however, to some extent, support Majumdar's (1955) theory that the axillant leaf and the axillary bud may represent the two forks of a dichotomous branching of an axis.

The origin of axillary buds reported in *Galium*, *Oldenlandia*, *Dentella*, *Stephagyne*, *Coffea bengalensis*, and particularly in *Coffea arabica*, are very interesting. The twin buds, foliar and foliar, axial and axial, and foliar and axial, and their vascular supply appear to point to the identical nature of the foliar and axial buds. Their origin, position, with reference to one another and their vascularization point to the following conclusions :

(i) The leaf and the axis, both of which bear the axillary buds, are equivalent organs. This lends support to Arber's (1950) partial-shoot theory of the leaf with 'an inherent urge towards the development of whole-shoot character' (p. 78). Recently Wardlaw (1949, 1957) and Cutter (1956) have shown that bud could be induced in a leaf-site, and an undetermined leaf primordium could be transformed into a bud.

(ii) In many species the bud initiation takes place in the tissue at the junction of the collar and the axis before the former separates from the latter. The collar then separates from the axis, and during the process if the line of separation passes external to the bud primordium it becomes axial, and if internal to it, it becomes foliar. In some species a separation zone organises surrounding the bud primordium to separate it both from the collar and the axis.

(iii) The buds appear to be twin products of ultimate dichotomy of an axis.

(iv) The foliar buds normally do not develop into branches because of their position and vascularization to which may be added the hereditary factor controlling the system of branching in these species.

(v) The axillary buds originate in the vacuolating dividing cells of the ground tissue. This is supported by Vöchting (1874), Priestley and Swingle (1929), Majumdar (1942), Sharman (1942), and Kundu and Rao in unbranched jute (1954), though the majority of investigators reported their origin from 'detached meristem'.

(vi) The branch buds, whether axial or foliar in origin, always receive their vascular supply from the central stele, whereas the foliar buds which are mostly ephemeral, are without any vascular supply, or get supplies from the median bundle of the leaf-trace, or secondarily from the axial bud-trace.

CONCLUSION

1. The collar of the Rubiaceae represents the united bases of the leaves, two or three as the case may be. It has two parts : one or the lower included in the axis as its outer component, and the other free from it and grows upwards in the form of a tubular sheath.

2. The collar is differentiated into two regions : the *petiolar regions* which contain the median bundle of the leaf-trace or its equivalent, and is radially extended; and the *interpetiolar regions* forming its lateral parts which generally contain the laterals and/or their branches.

3. The sheat' ing leaf-base or the free collar extends up to the region of separation of the petiole which is continuous or the prolongation of the petiolar regions (*soubassements foliares* of Gregoire, 1935) free from the collar.

4. The interpetiolar regions of the collar in continuation with the adaxial strip of the petiolar region after the separation of the petiole grows upwards in the form of a connate stipule, as in *Gardenia* spp. No inter-or intra-petiolar stipules are formed in this case.

Or, the collar may grow into a pair of interpetiolar stipules immediately after the separation of the petiole from it, or the collar may grow upwards as a sheathing base before it separates into a pair of interpetiolar stipules, or the collar by local outgrowth under the influence of a composite bundle may give rise to the stalked foliaceous stipules of the genus *Rubia*.

5. The stipules, connate or interpetiolar, receive their vascular supply from the laterals and/or their branches. Their development and vascularization have been followed and described in detail.

6. The Rubiaceous species are unique in having both foliar and axial bud primordia developed in the same parent bud, sometimes at different nodes, and in some cases at the same node in the axil of the same leaf. The foliar buds are formed near the apex and are ephemeral whereas the axial buds which normally develop into branches originate a few nodes below the growing point, and get their vascular supply directly from the axial cylinder.

Another unique feature has been noticed and reported in the previous pages : the bud primordium originates in the ground tissue at the junction of the collar and the axis, it separates from both by the formation of a separation zone round it. For other points of interest see under The Axillary Bud.

REFERENCES

Arber, A. (1950). The Natural Philosophy of Plant Form. Cambridge.
Boke, N. R. (1944). Histogenesis of the leaf and areole in *Opuntia cylindrica*. *Amer. J. Bot.,* **31**, 299–316.
———— (1951). Histogenesis of the vegetative shoot in *Echinocereus*. *Ibid.*, **38**, 23–38.
———— (1955). Development of the vegetative shoot in *Rhipsalis cassytha*. *Ibid.*, **42**, 1–10.
Cutter, E. G. (1956). Experimental and analytical studies of Pteridophytes. XXXIII. The experimental induction of buds from leaf primordium in *Dryopteris aristata* Druce. *Ann. Bot.* N.S., **20**, 143–165.
Esau, K. (1943). Origin and development of primary vascular tissues in seed plants. *Bot. Rev.*, **9**, 125–206.
———— (1953). Plant Anatomy. John Wiley & Sons, Inc. New York.
———— (1954). Primary vascular differentiation in plants. *Biol. Rev.*, **29**, 46–86.

Ganong, W. F. (1894). Beitrage zur etc. *Flora*, **79**, 49–86 (in Boke, 1955).

Garrison, R. (1949*a*). Origin and development of axillary buds in *Syringa vulgaris* L. *Amer. J. Bot.*, **36**, 205–213.

———— (1949*b*). Origin and development of axillary buds in *Betula papyrifera* Marsh. and *Euptelea polyandra* Sieb. *Ibid.*, **36**, 379–89.

———— (1955). Studies in the development of axillary buds. *Ibid.*, **42**, 257–66.

Gifford, E. M. Jr. (1951). Ontogeny of the vegetative axillary buds in *Drimys winteri* var. *chilensis. Ibid.*, **38**, 234–243.

Goebel, K. (1899). Pflanzenbiologische etc. I. Teil. Marburg. (in Boke, 1955).

———— (1905). Organography of Plants. II. Eng. ed. Oxford.

Grégoire, V. (1935). Données nouvelles sur la morphogénèse de l'axe feuille dans les Dikotylees. *C. R. Acad. Sci. Paris*, 200.

Heinig, R. L. (1899). Glossary of Botanic terms. Calcutta.

Hofmeister, W. (1851). Vergb. etc. (in Saunders, 1922).

Hsü, J. (1944). Structure and growth of the shoot apex of *Sinocalamus Beechyana. Amer. J. Bot.*, **31**, 404–411.

Koch, L. (1893). Die vegetative Verzweigung etc. *Jb. Wiss. Bot.*, **25**, 380–488. (in Esau, 1953)

Kundu, B. C. and Rao, N. S. (1952). Origin and development of axillary buds in jute. *Nature, Lond.*, **170**, 1128.

———— (1954). Origin and development of axillary buds in jute (*Corchorus capsularis*). *Ann. Bot. N. S.*, **18**, 367–75.

———— (1955). Origin and development of buds in *Hibiscus canabinus. Amer. J. Bot.*, **42**, 830–37.

———— (1957). The shoot apex of *Boehmeria nivea* during morphogenesis. *La Cellule*, **58**, 219–28.

Lodin, R. B. (1954). Vegetative shoot apex of *Zea mays. Amer. J. Bot.*, **41**, 11–17.

Leinfellner, W. (1937). Beitrage zur etc. *Z. Bot.*, **86**, 1–60. (in Boke, 1955).

Louis, J. (1935). L'Ontogenese du systeme conducteur dans la pousse feuille des Dicotylées et Gymnospermes. *La Cellule*, **44**, 87–102.

Majumdar, G. P. (1942). The organisation of the shoot in *Heracleum* in the light of development. *Ann. Bot. N. S.*, **6**, 49–82.

———— (1949). Leaf development at the growing apex and phyllotaxis in *Heracleum. Proc. Indian Acad. Sci.*, **28**(B), 83–98.

———— (1955*a*). The complete foliage leaf. *Ibid.*, **42**(B), 65–72.

———— (1955*b*). The foliage leaf and axillary bud. *Trans. Bose Res. Inst.*, **20**, 87–93.

———— (1956). Stipules, stipels, ligules and leaf-sheath. *Proc. Indian Acad. Sci.* **43**(B), 9–22.

———— (1957). The shoot of higher plants : Its morphology and phylogeny, *J. Asiat. Soc. Beng.*, **23**, 139–62.

———— and Dutta, A. (1946). Developmental Studies : I. Origin and development of axillary buds with special reference to two dicotyledons. *Proc. Indian Acad. Sci.*, **23**(B), 249–59.

Majumdar, G. P. and Mitra, G. C. (1948). The origin and development of stipules in *Morus alba* Linn. *Bull. bot. Soc. Bengal*, **2**, 1–8.

Majumdar, G. P. and Pal, P. K. (1958*a*). The interpetiolar stipules of *Galium mollugo. Proc. Indian Acad. Sci.*, **48**(B), 211–222.

———— (1958*b*). The stipules of the Rubiaceae : A Review. *Trans. Bose. Res. Inst.*, **22**, 57–68.

———— (1959). The stipules of the Rubiaceae. II. Stipules of *Anthocephalus cadamba* Miq., *Hamelia patens* Jac. and *H. sphaerocarpa* Rui & Pav. *Bull. bot. Soc. Bengal, Agharkar Comm. Vol.* (in press).

Metcalfe, C. R. and Chalk, L. (1950). Anatomy of the Dicotyledons. II. Oxford.

Miller, H. A. and Wetmore, R. H. (1946). Studies in the developmental anatomy of *Phlox drummondii* Hook. III. The apices of mature plant. *Amer. J. Bot.*, **33**, 1–10.

Mitra, G. C. (1945). The origin, development and morphology of the ochrea of *Polygonum orientale. J. Indian bot. Soc.*, **26**, 191–200.

———— (1948). Developmental Studies. The interpetiolar stipules of Rubiaceae with special reference to *Paederia foetida* and *Ixora parviflora. Ibid.*, **27**, 150–66.

———— (1949). Comparative account of the development of the base in the sheathing, the stipulate and the exstipulate leaves of four species of dicotyledon. *Bull. bot. Soc. Bengal*, **3**, 33–43.

———— (1952). The origin and development of vegetative axillary buds in dicotyledons. *Proc. 39th. Indian Sci. Congr.*, III, pp. 27–28.

Mitra, G. C. and Majumdar, G. P. (1952). The leaf-base and the internode : Their true morphology. *Palaeobotanist*, **1**, 351–367.

Parkin, J. (1948). The stipule considered phylogenetically. *Northw. Nat.*, 63–82, Mar.-Dec. England.

Philipson, W. R. (1949). The ontogeny of shoot apex in dicotyledons. *Biol. Rev.*, **24**, 21–50.

Priestley, J. H. and Swingle, C. F. (1929). Vegetative propagation from the standpoint of anatomy. *Dep. Bull. U. S., Dep. Agric.*, 151.

Reeve, R. N. (1943). Comparative ontogeny of the inflorescence and the axillary vegetative shoot in *Garrya elliptica*. *Amer. J. Bot.*, **30**, 609–619.

Saha, B. (1954). The shoot apex of *Oryza sativa*. *The Scientists Pakistan*, **2**, 9–18. Karachi.

Saunders, E. R. (1922). The leaf-skin theory of the stem. *Ann. Bot.*, **36**, 135–65.

Sharman, B. C. (1942). Developmental anatomy of the shoot of *Zea mays*. *Ann. Bot.* N. S., **6**, 245–82.

———— (1945). Leaf and bud initiation in the Gramineae. *Bot. Gaz.*, **106**, 269–89.

Sifton, H. B. (1944). Developmental morphology of vascular plants. *New Phytol.*, **43**, 87–123.

Sinnott, E. W. and Bailey, I. W. (1914). Investigation on the phylogeny of Angiosperms. III. Nodal anatomy and the morphology of Stipules. *Amer. J. Bot.*, **1**, 302–322.

Sterling, C. (1949). The primary body of the shoot of *Dianthera americana*. *Ibid.*, **36**, 184–93.

Strasburger's Text Book of Botany. (1930). Eng. ed. London.

Vöchting, H. (1873–1874). Beitrage zur Morphologie etc. *Jb. Wiss. Bot.*, **9**, 327–484. (in Boke, 1955).

Wardlaw, C. W. (1949). Experiments on organogenesis in ferns. *Growth* (Suppl.) **9**, 93–131.

———— (1950). The comparative investigation of apices of vascular plants by experimental studies. *Phil. Trans.*, *B* **234**, 583–604.

———— (1952). Phylogeny and Morphogenesis. London.

———— (1957). On the organization and reactivity of the shoot apex in vascular plants. *Amer. J. Bot.*, **44**, 176–185.

Warming, E. (1872). Forgreningsforhold etc. (in Arber, 1950).

White, D. J. B. (1955). The architecture of the stem apex and the origin and development of the axillary buds in seedlings of *Acer pseudoplatanus* L. *Ann. Bot.* N.S., **19**, 437–49

Willis, J. C. (1951). A Dictionary of Flowering Plants and Ferns. Cambridge.

Abbreviations used :

ax. axis; *axb.,* axial bud; *bd.,* bud; *br.,* branch; *btr.,* bud-trace; *brb.,* branch bundle of the leaf-trace; *cb.,* composite bundle; *col.,* collar; *fb.,* foliar bud; *gl.,* gland; *int. st.,* interpetiolar stipule; *issb.,* inner series of secondary bundles; *L.,* lateral leaf-trace bundle; L_1, L_2, *a, b, c.,* branches of *L.; Lf. lf.,* leaf; *M.,* median trace bundle; *p.,* petiole; *sep.,* separation of the collar from the axis; *sp.,* spine; *sry. bud,* secondary bud; *sst.,* sheathing stipule; *st.,* stipule; *stb.,* base of the stipule; *st. tp.,* stipule tip; *trb.,* leaf-trace bundle; *sz.,* separation zone.

COMPARATIVE EFFECTS OF YEAST, VITAMIN B COMPLEX, AND VITAMIN B_{12} ON THE SURVIVAL RATE OF INDIAN MINOR CARP DURING THE FIRST TWO WEEKS OF LIFE

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ABSTRACT

To compare the effect of yeast, vitamin B complex, and vitamin B_{12} on the survival rate of Indian minor carp (*Labeo bata, Cirrhina reba, Crossocheilus latia*), one day old carp were randomly allocated to thirty experimental units. Using a randomized block design, each unit was assigned to one of six treatments: control, B_{12}, B complex, yeast, yeast with B_{12}, B complex with B_{12}. B_{12} was given in tablets containing $25\mu g$ crystalline B_{12}; B complex in tablets containing 10 mg. thiamine hydrochloride, 3 mg. riboflavin, 2 mg. pyridoxine hydrochloride, 15 mg. nicotinic acid amide, and 3 mg. calcium pantothenate; and yeast in tablets containing 0.44 gm. dehydrated Brewer's yeast, 0.06 mg. thiamine hydrochloride, 0.03 mg. riboflavin, 0.15 mg. niacin, 0.23 gm. protein, 0.33 gm. carbohydrate, 0.02 gm. fat, and 0.03 gm. ash. Each experimental unit contained 9 litres of water and received 1 tablet of each of its assigned substances daily for 14 days. After 14 days, 18 per cent in the untreated control survived; 36 per cent receiving B_{12} only survived; and 78 to 80 per cent survived in treatments containing yeast or B complex. Differences between treatments were significant ($P<.01$). Survival rates for all treatments were significantly greater than that of the control, and treatments with B complex or yeast significantly greater than treatment with B_{12} alone. Treatments including yeast or B complex did not differ significantly from each other.

An important part of the diet in northeast India is provided by fresh-water fishes native to the region. Among the more popular fishes are the major and minor species of Indian fresh-water carp. The major species include *Catla catla, Cirrhina mrigala,* and *Labeo rohita,* while the minor species include *Labeo bata, Labeo boga, Labeo dero, Cirrhina reba, Crossocheilus latia,* and others (Shaw and Shebbeare, 1937).

In commerical carp cultivation, newly hatched fish are caught at the river spawning grounds and transported to large ponds. The fish are transported in earthen vessels in which they are kept for several days. The period immediately following hatching, when the fish are transferred to ponds, is characterized by a high incidence of mortality. Mortality is highest during the first few days, after which it gradually declines. Experiments in this laboratory show that, for some major species of carp, treatment with the B vitamins significantly enhances survival in this period. It may be that during the stage of most rapid growth which occurs after hatching, the young fish are particularly susceptible to deficiencies, which may be counteracted by vitamin supplements.

The role of vitamins in enhancing survival is suggested by data collected previously in this laboratory. Keeping the newly hatched fish in earthen vessels without further treament, 4 to 22 per cent survive. Experimental treatments significantly enhancing survival, in which 35 to 73 per cent survive, are briefly indicated below:

1. Vitamin B complex, including B_{12}, nicotinamide, riboflavin, calcium pantothenate, and pyridoxinehydrochloride, when *Cirrhina mrigala* is the predominant species (Das and Krishnamurthy, 1958*a*);

2. Vitamin B_{12}, when *Labeo rohita* is the predominant species (Das **and** Krishnamurthy, 1958*b*);

3. Cobalt nitrate mixed with an extract from goat stomachs, as effective as B_{12} at less than 1 per cent of the cost, for *Catla catla, Cirrhina mrigala* and *Labeo rohita* (Das 1959).

To determine whether minor species of carp would also benefit from vitamin treatment during this early period, and whether their requirements would be different from those of the major carp, the present experiment was carried out, comparing the effectiveness of yeast, vitamin B complex and vitamin B_{12}.

DESIGN OF THE EXPERIMENT

A randomized block design was adopted for five blocks and six treatments, making a total of thirty experimental units. Each treatment was replicated five times, once in each of the five blocks. Within each block, the six treatments were randomly ordered. The six treatments, their symbols and dosages, were as follows :

'mbol	Treatment	Dosage
A	Control	——
B	Vitamin B_{12}	25 µg.
C	Vitamin B complex	1 tablet
D	Yeast	1 tablet
E	Yeast and B_{12}	1 tablet & 25 µg.
F	B complex and B_{12}	1 tablet & 25 µg.

Counts of the number of fish dying were taken daily for fourteen days, and a count of the number of fish alive after that period was over was also taken. Water temperature and pH were recorded daily for each experimental unit. Fluorescent tube lights were arranged to ensure equal lighting throughout the laboratory. Fans were arranged for constant circulation of air and to maintain a constant temperature in the laboratory.

MATERIALS AND METHODS

One day old minor carp (*Labeo bata, Cirrhina reba,* and *Crossocheilus latia*) were procured from the same source. A sampling procedure was adopted to allocate the carp to the experimental units, as their exact enumeration was not feasible due to their minute size. One tea-spoon of carp, in pond water, was assigned randomly to each experimental unit. There were thirty experimental units, each consisting of an earthen bowl 16" in diameter, containing 9 litres of water, and covered with screening. Throughout the experimental period, the water of each experimental unit was changed every 24 hours to prevent accumulation of waste products, and fresh pond water obtained from the same source. The water was maintained at 9 litres for each experimental unit. Water temperature ranged from 26.5°C to 28.3°C and pH from 7.6 to 8.5 throughout the experimental period.

The carp were fed live Daphnia from the same source, given 10 c.c. by volume each day to each experimental unit. Daily checks revealed that some Daphnia were always found remaining, so this ration was considered sufficient.

To obtain the experimental counts, the dead carp were completely enumerated each day at the same time. The dead carp were withdrawn from the experimental unit with a glass pipette, after inducing a centrifugal current in the water, placed on blotting paper and counted. A fresh pipette was used for each experimental unit each day. The total number of dead carp, plus the number alive at the end of the experiment, reconstructed the initial number placed in the experimental unit.

The dosage per 9 litres of water for the different treatments was as follows :
　　1.　Each experimental unit belonging to treatments B, E and F received one 25μg. tablet of crystalline vitamin B$_{12}$ (British Drug Houses) daily ;
　　2.　Each experimental unit belonging to treatments C and F received one vitamin B complex tablet (Teddington Chemical Factory, Bombay) daily of the following composition : 10 mg. thiamine hydrochloride, 3 mg. riboflavin, 2 mg. pyridoxine hydrochloride, 15 mg. nicotinic acid amide, and 3 mg. calcium pantothenate ;
　　3.　Each experimental unit belonging to treatments D and E received one yeast tablet (Squibb) daily containing 0.44 gm. dehydrated Brewer's yeast. Each tablet supplies 0.06 mg. thiamine hydrochloride, 0.03 mg. riboflavin, and 0.15 mg. niacin. The experimental dosage was given at the same time daily.

RESULTS

　　Table 1 presents the total number alive on each day, pooled over the five replications, and the cumulative survival rate, for the six treatments. For any day, the cumulative survival rate is the number alive on that day for all five replications divided by the initial number of those replications. The cumulative survival rates are presented graphically in Figure 1.
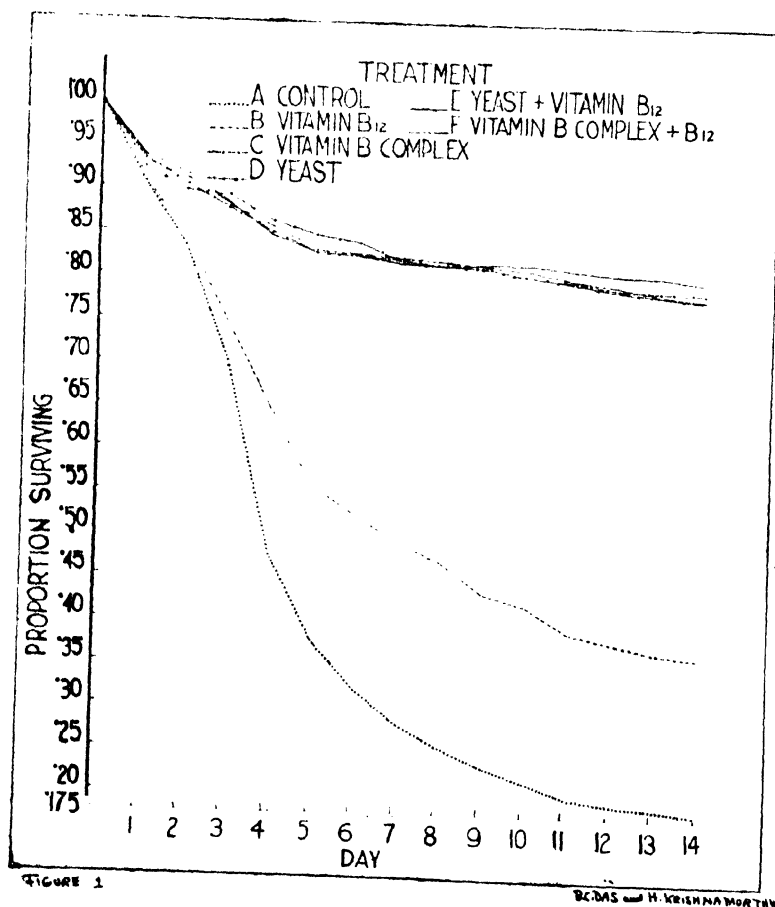

TEXT-FIG. 1.
Survival Rates of Indian Minor Carp Under Different Experimental Treatments.

To determine whether the treatments differed significantly, an analysis of variance was carried out on the final proportion surviving for the thirty experimental units. The proportions are given in Table 2 and the analysis of variance in Table 3. The critical difference between two treatment means required to obtain 't' at the 1 per cent level of confidence was computed (see Table 2)*. In Table 2, the mean for each treatment, and difference between treatment and control means, have been given for comparison with the critical difference. The survival proportions are presented graphically in Figure 2.
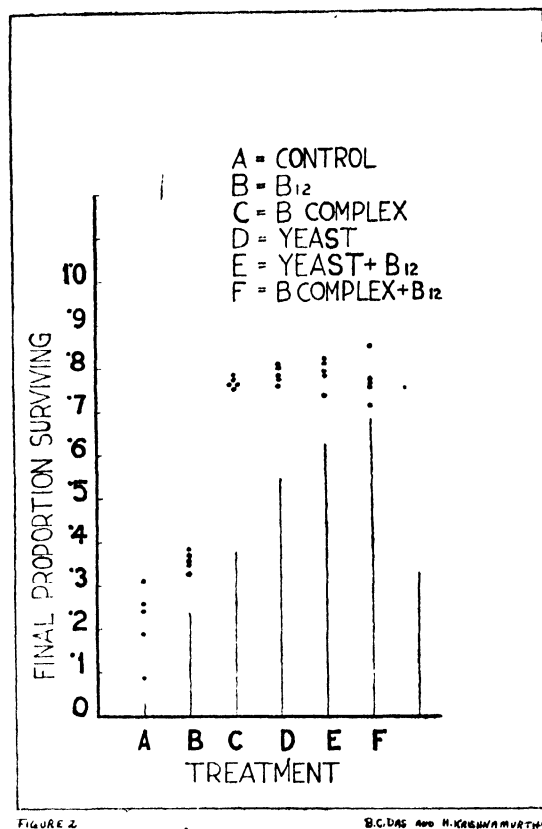

TEXT-FIG. 2.

Final Proportion Surviving of All Experimental Units Under Different Treatments.

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* For 2 treatments, the critical difference for the means is

$$\left(t_{1\%} \right)\left(\text{s.e.}\sqrt{1/n_1 \times 1/n_2} \right)$$

with $t_{1\%}$ being the value of t required for significance at the 1% level for the degrees of freedom of the error mean square in the analysis of variance summary table, s.e. being the square root of the error mean square, and n_1 and n_2 being the number of replications in the two treatments.

4

TABLE 1

Cumulative Survival Rates for All Experimental Treatments

	Treatment					
	A	B	C	D	E	F
Day	Control	B_{12}	B Complex	Yeast	Yeast $+ B_{12}$	B Complex $+ B_{12}$
1	.9065	.9116	.9231	.9331	.9363	.9251
2	.8361	.8391	.8988	.9044	.9148	.9080
3	.7190	.7443	.8808	.8798	.8827	.8812
4	.4777	.6564	.8606	.8511	.8479	.8559
5	.3804	.5598	.8462	.8322	.8327	.8352
6	.3251	.5172	.8359	.8255	.8239	.8276
7	.2837	.4923	.8223	.8221	.8194	.8214
8	.2574	.4683	.8168	.8143	.8155	.8161
9	.2364	.4379	.8091	.8093	.8144	.8108
10	.2203	.4229	.7987	.7998	.8123	.8067
11	.1994	.3939	.7940	.7948	.8095	.7984
12	.1934	.3821	.7884	.7904	.8039	.7904
13	.1897	.3694	.7851	.7870	.8011	.7851
14	.1811	.3586	.7763	.7845	.7965	.7787

TABLE 2

Initial Number and Final Proportion Surviving for All Experimental Units

	Treatment											
	A Control		B B_{12}		C B Complex		D Yeast		E Yeast $+B_{12}$		F B Complex $+B_{12}$	
Replication	IN†	FP††	IN	FP	IN	FP	IN	FP	IN	FP	IN	FP
1	739	.0893	530	.3340	438	.7854	645	.8016	476	.7920	652	.8466
2	197	.2538	559	.3846	756	.7659	962	.7640	553	.7414	878	.7745
3	379	.1900	369	.3523	444	.7883	530	.7849	564	.8245	491	.7719
4	218	.3119	376	.3511	683	.7745	697	.7776	866	.8152	542	.7675
5	328	.2470	372	.3683	397	.7758	753	.8021	381	.7979	830	.7410
Mean Proportion	.2184		.3581		.7780		.7860		.7942		.7803	
Difference from Control			.1397**		.5596**		.5676**		.5758**		.5619**	
Difference from B_{12}					.4199**		.4279**		.4361**		.4222**	

1% Critical Difference = .0729

†IN = Inital Number
††FP = Final Proportion
**P < .01

TABLE 3

Analysis of Variance Summary Table

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F
Between Treatments	1.6923	5	.3385	199.1176**
Error	0.0415	24	.0017	
Total	1.7338	29		

**P < .01

To determine whether within treatment proportions differed significantly, a homogeneity chi square was computed for each treatment. The values of chi square are given in Table 4. To test the hypothesis that significant within treatment variation could be attributed to differences in initial number, correlations between initial number and final proportion surviving were computed for those treatments having a significant value of chi square. The correlations are also given in Table 4.

TABLE 4

Test of Relationship Between Initial Number and Final Proportion Surviving

	Treatment	Homogeneity Chi Square †	Correlation
A	Control	94.56**	—.9442*
B	B_{12}	4.68	
C	B Complex	1.06	
D	Yeast	6.56	
E	Yeast $+B_{12}$	15.00**	.2741
F	B Complex $+B_{12}$	11.46*	—.2055

*P < .05
**P < .01
†4 degrees of freedom

DISCUSSION

Curves for the survival rate of Indian minor carp during the first two weeks of life are presented in Figure 1. For the control condition, the curve initially drops rapidly, after which the rate of descent decreases. Under vitamin B_{12} treatment, the curve shows a less steep descent, and does not fall to the level of the control curve. The curves obtained for treatments with yeast or B complex show a very slight descent, and level off within a few days. The control curve is similar to those obtained for major species of carp under untreated control conditions.

The effectiveness of vitamins in enhancing survival during the early period of life is confirmed by the results of this experiment on Indian minor carp. Examination of Figure 2 shows that treatments with yeast and with vitamin B complex were definitely superior to the control and also to treatment with vitamin B_{12} alone. Treatment with vitamin B_{12} alone was superior to the control. The four treatments with yeast and B complex did not differ significantly from each other. For treatments with B complex or yeast, 78 to 80 per cent survived, as compared to 18 per cent in the control and 36 per cent when only B_{12} was given. These results are confirmed statistically by the analysis of variance given in Table 3 and the differences between treatment means given in Table 2.

In this experiment, using minor carp, B complex without B_{12} was more effective in enhancing survival than B_{12} alone. This suggests that the vitamin requirements of the minor carp differ from those of the major carp indicated by previous experiments. For both major and minor species, the B vitamins as a group apparently have an important role to play during the early period of life. In this experiment, treatment with B complex or yeast resulted in 78 to 80 per cent survival, as compared to 18 per cent in the untreated control condition. A saving of 60 per cent suggests that this vitamin treatment can be recommended as a general treatment for carp cultivation on a commercial scale. In this connection, the relative price of yeast and B complex should be considered. As yeast is the cheaper of the two, it would be more likely to be adopted on a commercial scale.

The period during which treatment is most effective is also suggested by these data. The cumulative survival rates in Table 1 show that the period of highest mortality occurs during the first seven days for the control group. For the most effective treatments, protection is afforded during this period of high mortality, resulting in a higher final proportion surviving. This result is in agreement with the results of previous experiments, and suggests that treatment during the first week would effectively reduce mortality of the young carp.

The final proportion surviving is also shown to be influenced by the initial number present, but this effect is noted only in the case of the control group. This influence was examined in the following manner : first, the homogeneity of the five replications within each treatment was tested. The results given in Table 4 show that for three treatments, the hypothesis of homogeneity could be rejected. For these three treatments, the correlations between initial number and final proportion surviving were computed. The only significant correlation ($P<.05$) was found for the control group : for the other two treatments, the correlations were insignificant. Thus, in the control group, as initial number increased, survival rate decreased. This effect may be attributed to overcrowding when initial number is high. For the control group, 9 per cent survived at the highest density (initial number 739) while 25 per cent survived at the lowest density (initial number 197). These results are also in agreement with those of earlier experiments, i.e. initial number is inversely related to survival for the untreated control, but not for significant treatments. It seems that vitamin treatment may counteract the influence of initial number on the final proportion surviving.

SUMMARY

1. The survival rate of Indian minor carp (*Labeo bata*, *Cirrhina reba*, and *Crossocheilus latia*) was studied during the first two weeks following hatching under a no-treatment control condition and the following treatment conditions : vitamin B_{12} vitamin B complex, yeast, yeast with B_{12}, and B complex with B_{12}.

2. Survival was significantly enhanced by the experimental treatments. In the untreated control condition, 18 per cent survived ; for B_{12} alone, 36 per cent survived ; and in treatments with yeast or B complex, 78 to 80 per cent survived.

3. Mortality was found to be highest during the first week in the untreated control condition. During this period, experimental treatments effectively reduced mortality.

4. Final proportion surviving was shown to be significantly and inversely related to the initial number for the untreated control, but not for the treated groups.

5. These data suggest that treatment with yeast or vitamin B complex may be of commercial value.

REFERENCES

Das, B. C. (1959). Comparative effects of vitamin B_{12}, cobalt nitrate, and ruminant stomach extract on the survival rate of Indian carp during the first three weeks of life. *Indian J. Fish.* 6, 211–221.

Das, B. C. and Krishnamurthy, H. (1958a) Survival rates of the Indian carp (*Catla catla, Labeo rohita, Cirrhina mrigala*) from first to fourth week of life under different experimental treatments. *Sankhyā* (in press).

——— (1958b). Survival rates of Indian carp (*Catla catla, Labeo rohita, Cirrhina mrigala*) from first to fourth week of life under experimental treatments isolating vitamin B_{12} from vitamin B complex. *Ibid* (in press).

Shaw, G. E. and Shebbeare, E. O. (1937). The fishes of northern Bengal. *J. Asiat. Soc. Beng. Sci.* 3 (whole volume).

Snedecor, G. W. (1956). Statistical methods. Fifth Edition. Ames, Iowa State College Press

EFFECT OF POTASSIUM CHLORIDE ON NITRATE FORMATION IN HUMUS RICH SOIL BY THE APPLICATION OF NITROGENOUS COMPOUNDS*

by S. K. Ghosh, *State Agricultural Research Institute, Calcutta*
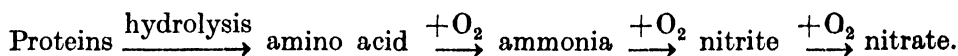
(Communicated by S. Ghosh, F.N.I.)

Abstract

1. There is much loss of nitrogen when ammonium sulphate or urea undergo nitrification on the soil surface due to the formation and decomposition of the unstable product ammonium nitrite and hence nitrate formation is very less. Nitrate formation is, however, prominent at lower concentrations of nitrogen.

2. Potassium chloride added to the soil along with ammonium sulphate or urea increases nitrate concentration due to the formation of potassium nitrite which being more stable than ammonium nitrite remains in the system and can slowly oxidise into potassium nitrate. Again the presence of potassium chloride in the solution phase increases the ionic strength of the medium and checks the decomposition of ammonium nitrite due to secondary salt effect.

It has been observed by Dhar (1951) and Ghosh (1953) that when ammonium salts, urea, uric acid, oil cakes, proteins etc. are allowed to undergo slow oxidation in air in presence of soil surface or in presence of surfaces like SiO_2, Fe_2O_3, MnO_2 etc. the following changes take place.

$$\text{Proteins} \xrightarrow{\text{hydrolysis}} \text{amino acid} \xrightarrow{+O_2} \text{ammonia} \xrightarrow{+O_2} \text{nitrite} \xrightarrow{+O_2} \text{nitrate.}$$

These changes are oxidation processes which are accelerated by increased aeration, absorption of solar light and by an increase of temperature. Thus under these conditions at ordinary temperature the whole of ammonia cannot be readily converted into its oxidised products and hence ammonium ion and occasionally free ammonia have to co-exist with nitrite ion or nitrous acid, nitrate ion or nitric acid and hence marked decomposition takes place with liberation of nitrogen gas. An unstable intermediate compound ammonium nitrite is formed during this process which can undergo both oxidation and decomposition as follows :

$$2NH_4NO_2 + O_2 = 2NH_4NO_3 \qquad \qquad .. \quad (i)$$

$$NH_4NO_2 \qquad = N_2 + 2H_2O + 718 \text{ cals} \qquad .. \quad (ii)$$
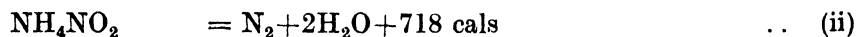
The second chemical change is more prominent than the first as it is highly exothermic and hence there is a considerable loss of nitrogen from soil than the formation of nitrate when it is manured with all types of nitrogenous compounds. The temperature coefficient of the decomposition of ammonium nitrite has been shown by Arndt (1901) to be of the order of 3 for a 10°C rise of temperature so that the reaction seems to be prominent in tropical country soils. Again the decomposition of ammonium nitrite is an autocatalytic process and is markedly accelerated by acids. Hence, the loss of nitrogen also becomes prominent in cold countries, where the soils have a tendency to be acidic and specially in soils not containing much calcium carbonate or calcium phosphate. The researches of

Lipman and Blair (1921), Russell and Richards (1917), Shutt (1910) and others have shown that nitrogen in gaseous state is lost from soils when the conditions are favourable for oxidation.

Field trials have shown that the recovery of nitrogen by crops never exceeds 50 per cent as has been stated by Russell (quoted by Dhar 1951) by adding ammonium sulphate at the rate of 1 cwt. per acre. Recovery of phosphates and potash may however go up to 86 per cent. Lohnis and Fred (1923) have reported the following recovery in field experiments lasting for 4 years.

Nitrogen	P_2O_5	K_2O
7.8 to 46.1%	10.1 to 75.6%	22.4 to 85.1%

It is clear, therefore, that nitrogen loss is more prominent than the formation of nitrates when ammonium salts and other nitrogenous compounds undergo nitrification on the soil surface. Dhar and Ghose (1955) have, however, observed that potassium and calcium chloride markedly check the loss of nitrogen and increase nitrate formation during the nitrification of ammonium salts and urea on pure chemical surfaces as those of zinc oxide, iron oxide, etc.

In view of the above facts, effect of different doses of potassium chloride on nitrate formation in humus rich soil has been studied here using ammonium sulphate and urea as nitrogen rich compounds.

EXPERIMENTAL

100 grams of well dried and powdered humus rich soil (screened through a 100 mesh sieve) were accurately weighed and taken in shallow enamelled dishes. Amounts of urea or ammonium sulphate (A. R. quality), each containing exactly 1.00 gm. of nitrogen, were mixed with the soil with and without different doses of potassium as potassium chloride. The concentrations of potassium added were 2 gm., 5 gm., and 10 gm., per 100 grams of soil respectively. The contents of the dishes were thoroughly mixed and a few samples were taken out for the determination of their initial ammoniacal, nitrate and total nitrogen contents. Two sets of each mixture were made, one was kept exposed to light of an electric bulb of 60 watts working on 220 volts at a distance of 3 feet from the bulb whilst the other was kept covered with thick black cloth by the side of the exposed one. The mixtures were stirred carefully by means of a glass rod on alternate days and their moisture content was maintained at 25 per cent level by adding distilled water. The light exposure was continued day and night and samples were taken out at regular intervals for analysis of their ammoniacal, nitrate and total nitrogen contents. Total nitrogen was estimated by salicylic acid reduction method Treadwall and Hall, (1947) Ammoniacal nitrogen was determined by distilling a known qauntity of soil with magnesia and nitrate nitrogen by reduction with Devarda's alloy. The following experimental results were obtained.

TABLE 1

Percentage compoition of the oven dried soil sample

SiO₂	Sesquioxide	FO₂O₃	CaO	MgO	K₂O	P₂O₅	Total-C	Total-N	NH₃–N	NO₃–N
75.00	9.80	4.37	4.07	1.52	1.00	0.42	1.86	0.26785	0.00608	0.02035

pH of the soil = 8.00

TABLE 2

Average Temperature = 25°C
Light

Treatment	Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per 100 grams of the mixture		
		After 15 days	After 30 days	After 60 days
1. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$	NH_3—N = 0.96080	0.28570	0.17780	0.09850
	NO_3—N = 0.01940	0.04550	0.05880	0.08890
	Available—N = 0.98020	0.33120	0.23660	0.18740
	Total—N = 1.21077	0.53800	0.43850	0.40200
2. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$ + 2gm. K as KCl	NH_3—N = 0.92700	0.36360		
	NO_3—N = 0.01870	0.04940		
	Available—N = 0.94570	0.41300		
	Total—N = 1.16820	0.65410		
3. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$ + 5gm. K as KCl	NH_3—N = 0.88060	0.51080	0.38100	0.22230
	NO_3—N = 0.01780	0.05410	0.07140	0.10250
	Available—N = 0.89840	0.56490	0.45240	0.32480
	Total—N = 1.10960	0.77450	0.60670	0.55840
4. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$ + 10gm. K as KCl	NH_3—N = 0.81270	0.57140	0.44450	0.33340
	NO_3—N = 0.01640	0.06450	0.08333	0.12120
	Available—N = 0.82910	0.63590	0.52783	0.45460
	Total—N = 1.02410	0.91380	0.72970	0.68910

TABLE 3

Average Temperature = 25°C
Dark

Treatment	Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per 100 grams of the mixture		
		After 15 days	After 30 days	After 60 days
1. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$	NH_3—N = 0.96080	0.30770	0.20510	0.13333
	NO_3—H = 0.01940	0.04880	0.06250	0.09520
	Available—N = 0.98020	0.35650	0.26760	0.22860
	Total—N = 1.21077	0.59990	0.49670	0.44530
2. 100gm. Soil + 1.0gm N as $(NH_4)_2SO_4$ + 2gm. K as KCl	NH_3—N = 0.92700	0.38690		
	NO_3—N = 0.01870	0.05260		
	Available—N = 0.94570	0.43950		
	Total—N = 1.16820	0.72190		
3. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$ + 5gm. K as KCl	NH_3—N = 0.88060	0.54050	0.41600	0.26670
	NO_3—N = 0.01780	0.05880	0.07690	0.11112
	Available—N = 0.89840	0.59930	0.49290	0.37782
	Total—N = 1.10960	0.83860	0.67180	0.60111
4. 100gm. Soil + 1.0gm. N as $(NH_4)_2SO_4$ + 10gm. K as KCl	NH_3—N = 0.81270	0.60610	0.47620	0.37112
	NO_3—N = 0.01640	0.07140	0.09090	0.13333
	Available—N = 0.82910	0.67760	0.55710	0.50445
	Total—N = 1.02410	0.97690	0.78490	0.73800

TABLE 4

Average Temperature = 25°C
Light

Treatment	Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per per 100 grams of the mixture		
		After 15 days	After 30 days	After 60 days
1. 100gm. Soil + 1.0gm. N as Urea	NH_3—N = 0.00595	0.06667	0.10010	0.02222
	NO_3—N = 0.01987	0.02500	0.02670	0.04000
	Available—N = 0.02582	0.09167	0.12680	0.06222
	Total—N = 1.24120	0.48090	0.34000	0.29750
2. 100gm. Soil + 1.0gm. N as Urea + 2gm. K as KCl	NH_3—N = 0.00574	0.05333		
	NO_3—N = 0.01916	0.02860		
	Available—N = 0.02490	0.08193		
	Total—N = 1.19655	0.56390		
3. 100gm. Soil + 1.0gm. N as Urea + 5gm. K as KCl	NH_3—N = 0.00544	0.04000	0.08020	0.02500
	NO_3—N = 0.01818	0.03200	0.03333	0.05160
	Avainalbe—N = 0.02362	0.07200	0.11353	0.07660
	Total—N = 1.13520	0.67840	0.44630	0.35390
4. 100gm. Soil + 1.0gm. N as Urea + 10gm. K as KCl	NH_3—N = 0.00501	0.03100	0.07270	0.02860
	NO_3—N = 0.01674	0.04710	0.06650	0.07270
	Available—N = 0.02175	0.07810	0.13920	0.10130
	Total—N = 1.04580	0.79590	0.57960	0.43700

TABLE 5

Average Temperature = 25°C
Dark

Treatment	Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per 100 grams of the mixture		
		After 15 days	After 30 days	After 60 days
1. 100 gm. Soil + 1.0 gm. N as Urea	NH_3—N = 0.00595	0.08340	0.12000	0.03636
	NO_3—N = 0.01987	0.02670	0.02850	0.04450
	Available—N = 0.02582	0.11010	0.14850	0.08086
	Total—N = 1.24120	0.54690	0.39260	0.32720
2. 100gm. Soil + 1.0gm. N as Urea + 2gm. K as KCl.	NH_3—N = 0.00574	0.07272		
	NO_3—N = 0.01916	0.03100		
	Available—N = 0.02490	0.10372		
	Total—N = 1.19655	0.63570		
3. 100gm. Soil + 1.0gm. N as Urea + 5gm. K as KCl.	NH_3—N = 0.00544	0.05774	0.10020	0.04000
	NO_3—N = 0.01818	0.03480	0.03630	0.05710
	Available—N = 0.02362	0.09194	0.13650	0.09710
	Total—N = 1.13520	0.74520	0.49220	0.39160
4. 100gm. Soil + 1.0gm. N as Urea + 10gm. K as KCl.	NH_3—N = 0.00501	0.04450	0.09230	0.04450
	NO_3—N = 0.01674	0.05000	0.06900	0.08010
	Available—N = 0.02175	0.09450	0.16130	0.12460
	Total—N = 1.04580	0.86290	0.63690	0.47650

A perusal of the foregoing results shows that there is a considerable loss of nitrogen along with the formation of nitrate in the system when ammonium sulphate and urea are allowed to undergo oxidation on the soil surface. In the case of ammonium sulphate most of the nitrogen present is in ammoniacal form from the beginning. This ammoniacal form of nitrogen slowly undergoes oxidation on the soil surface and is converted into nitrate both in light and in the dark. This is evident from Tables 2 and 3 as the ammoniacal nitrogen slowly decreases from the system with an increase of nitrate nitrogen with lapse of time. During this process a considerable portion of nitrogen undergoes loss. For urea, however, there is very little nitrogen in the available form (sum of ammoniacal and nitrate nitrogen) in the beginning, but both ammoniacal and nitrate nitrogen appear after this nitrogenous compound remains in contact with the soil surface for sometime. Urea is first converted into ammonium carbonate on the soil surface showing an increase in the ammoniacal nitrogen content of the system in the first part of the experiment and finally this ammoniacal nitrogen is oxidised into nitrate nitrogen showing a decrease in the ammoniacal nitrogen content with an increase in the nitrate nitrogen in the second part of the experiment. This is evident from Tables 4 and 5. The efficiency of nitrate formation now requires a careful study. In the case of ammonium sulphate some of the ammoniacal nitrogen which has decreased from the system in a given time is converted into nitrate nitrogen and the efficiency of nitrate formation has been calculated from the amount of nitrate nitrogen formed per 100 grams of ammoniacal nitrogen which has decreased from the system. In the case of urea, however, ammoniacal nitrogen accumulates in the system after some time and then it is oxidised into nitrate nitrogen. Hence, in this case, the efficiency of nitrate formation has been calculated from the amount of nitrate nitrogen present per 100 grams of the available nitrogen present in the system in a given time. In Tables 6 and 7 results recording the efficiency of nitrate formation both from ammonium sulphate and urea in light and in the dark are given.

TABLE 6

Average Temperature = 25°C

Efficiency of nitrate formation from Ammonium sulphate

Concentration of K added as KCl	After 15 days		After 30 days		After 60 days	
	Exposed to light	kept in dark	Exposed to light	kept in dark	Exposed to light	kept in dark
1. nil	3.866	4.502	5.032	5.703	8.059	9.160
2. 2 gm.	5.449	6.276	—	—	—	—
3. 5 gm.	9.816	12.055	10.728	12.720	12.866	15.201
4. 10 gm.	19.933	26.612	18.177	22.139	21.865	26.479

A perusal of the foregoing tables shows that
(i) Efficiency of nitrate formation in the case of ammonium sulphate is more marked in the dark than in light. This is due to the fact that loss of nitrogen is more prominent in light than in the dark according to the mechanism of the loss of nitrogen stated before. As the ammoniacal nitrogen is slowly oxidised into nitrate nitrogen the efficiency of nitrate formation goes on increasing with lapse of time. It seems, therefore, that at lower concentrations of ammoniacal nitrogen

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TABLE 7

Average Temperature $= 25°C$

Efficiency of nitrate formation from urea

Concentration of K added as KCl	After 15 days		After 30 days		After 60 days	
	Exposed to light	kept in dark	Exposed to light	kept in dark	Exposed to light	kept in dark
1. nil	27.272	24.251	21.057	19.191	64.288	55.033
2. 2 gm.	34.908	29.888	.	...	...	—
3. 5 gm.	44.444	37.851	29.358	26.593	67.363	58.805
4. 10 gm.	60.307	52.921	47.773	42.777	71.767	64.286

its conversion into nitrate nitrogen is more prominent than the loss of nitrogen. The loss of nitrogen is more prominent in the first stage of the reaction than in the second stage of the reaction i.e. at higher concentrations of nitrogen than at lower concentrations of nitrogen. In the case of urea, however, the efficiency of nitrate formation is more marked in light than in the dark. This is due to the fact that accumulation of ammoniacal nitrogen and its final conversion into nitrate nitrogen is more marked in the light than in the dark. The efficiency of nitrate formation decreases in the first stage of the reaction but it increases in the second stage of the reaction. This is because accumulation of ammoniacal nitrogen takes place in the first stage of the reaction and it is oxidised into nitrate nitrogen in the second stage of the reaction.

(ii) Presence of potassium chloride in the system has increased nitrate formation both in the case of ammonium sulphate and urea in light as well as in the dark. In every case the greater the concentration of potassium chloride added, the greater is nitrate formation. This is due to the fact that potassium chloride reacts with the unstable substance ammonium nitrite formed in the system in the nitrification of ammonium sulphate and urea and form potassium nitrate, which being more stable than ammonium nitrite, remains in the system and can slowly oxidise into potassium nitrate. Again the presence of potassium chloride in the solution phase increases the ionic strength of the medium which is liable to check the decomposition of ammonium nitrite due to secondary salt effect according to the following equation (Bronsted 1928):

$$\log K - \log K_0 = -2.04\sqrt{\mu}$$

where μ is the ionic strength of the added neutral salt, K is the velocity constant in presence of a neutral salt, and K_0 is the velocity constant without the neutral salt. Dhar and Ghose (1955) have shown that calcium chloride in solution which provides greater ionic strength checks the loss of nitrogen and increases nitrate formation more effectively than potassium chloride solution of the same concentration using pure chemical oxides as surfaces. Gopala Rao (1939) has also noted that photosensitised oxidation of ammonia, using titanium oxide as surface, is retarded by traces of salts of colourless cations like Na^+, K^+, Ba^{++}, Sr^{++}, Al^{+++} etc. and that the greater the valency of cations, the more effectively they act as retarders. These points are further illustrated graphically in Figures 1 and 2.

It is clear, therefore, that the presence of potassium salts in the system should be able to minimise the amount of nitrogenous compounds added to the soil by

checking nitrogen loss and increasing nitrate formation and this is profitable from agricultural point of view.   In temperate countries due to the presence of large

FIG 1

FIG II
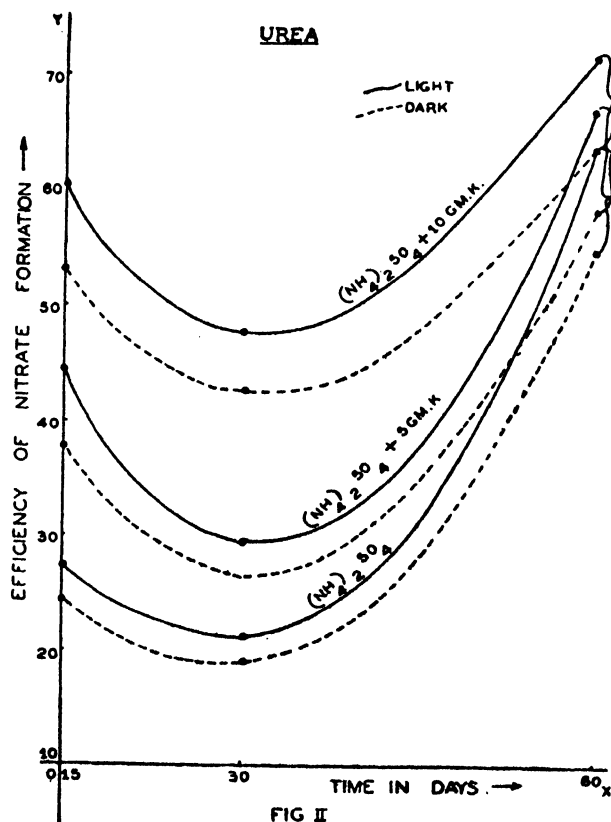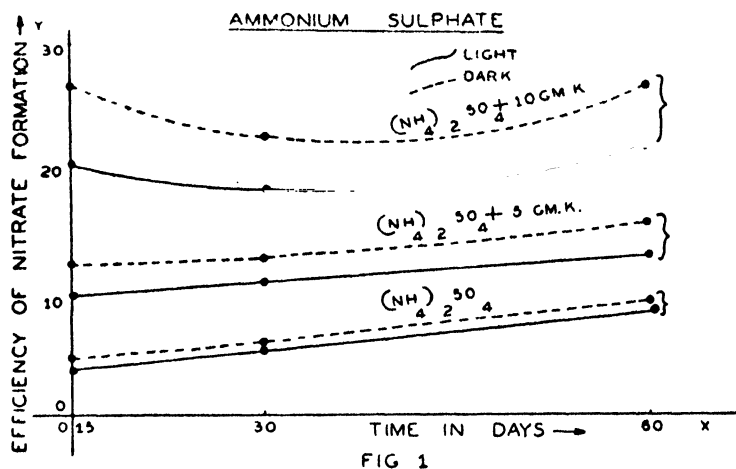
amount of humus the addition of ammonium salts leads to adorption of the majority of ammonium ions by the humus and the clay particles and therefore nitrogen may not be available to plants.   If to such a system potassium salts are added,

ammonium ions will be displaced by potassium ions from humus and the clay layer and thus will pass into solution phase to be readily nitrified for the use of crops. In such cases the addition of potossium salts may intensify the nitrogen effect and show positive interaction between potash and nitrogen as has been occasionally reported for potatoes at Rothamsted (Russel, 1950). For growing potatoes, beats and perhaps other tubers potash is of great value since these materials require large amount of potash for their growth. It seems, therefore, that in growing tubers potash may not only act as an intensifier of nitrogen effect but is also useful as a direct plant food.

ACKNOWLEDGEMENTS

REFERENCES

Arndt, K. (1901). *Z. phys. Chem.*, **39**, 64—90.
Bronsted, J. N. (1928). Acid and basic catalysis. *Chem. Rev.*, **5**, 231–338.
Dhar, N. R. (1951). Importance of organic manures and incrganic fertilisers. *Proc. nat. Acad. Sci. India*, A, **20**, Part IV, 151-192.
Dhar, N. R. and Ghosh S. K. (1955). Effect of potassium chloride and calcium chloride on nitrification and nitrogen loss from solutions of different nitrogen rich compounds. *Ibid*, A, **24**, Part III, 315-331.
Ghosh, S. K. (1953). Studies on the formation of Nitre Beds, D. Phil, Thesis, Alld. Univ. India.
Gopala Rao (1939). Photosensibilisierung durch feste stoffe. Titandioxye. *Z. phys. Chem.*, **184**A, 377.
Lipman, J. G. and Blair, A. W. (1921). Nitrogen losses under intensive cropping. *Soil Sci.* **12**, 1 19.
Lohnis F. and Fred, E. B. (1923). Text Book of Agricultural Bacteriology.
Russell, E. J. (1950). Soil Conditions and Plant growth, Longmans, Green and Co., London. VIII Edn., 63.
Russell, E. J. Quoted by Dhar, N. R. (1951).
Russell, E. J. and Richards, E. H. (1917). The changes taking place during storage of farm-yard manure. *J. agric. Sci.*, **8**, 495–563
Shutt. (1910). *Ibid.*, **3**, 335.
Treadwall, F. P. and Hall, W. T. (1947). Analytical Chemistry. John Wiley and Co. **2**, 493-94.

POPULATION STUDIES ON HADROPHANURUS* SPECIES (SCELIONIDAE : HYMENOPTERA), EGG PARASITE OF BAGRADA CRUCIFERARUM KIRK ON MAIZE** (ZEA MAYS) AT KARNAL

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Abstract

The control of insect pests by means of their parasites, predators and other natural enemies is one of the most economical and effective methods of control in applied entomology. The population studies of *Hadrophanurus species*, an egg parasite of *Bagrada cruciferarum* Kirk have, therefore, been studied. The rise and fall in the parasite population is directly proportional to the rise and fall in the population of the host. The maximum population of the parasite, i.e. 3881 adults per acre coincides with the maximum population of the host, i.e. 4939 eggs per acre. Similar interrelationship is also found when the population per acre of the host as well as that of the parasite are at their lowest level. There is no superparasitism in the species and its viability is as high as 100 per cent under field conditions. The percentage of parasitism was 85.1 per cent during the season. This high percentage of parasitism exterminates the host and proves fatal to the parasite as well.

Introduction

The control of pests by means of their parasites, predators and other natural enemies is one of the most economical and effective methods of control in applied entomology. In order, however, to utilise the method effectively the host parasite interaction, especially with reference to the population of insect pests and their natural enemies that constantly fluctuates, has to be studied in detail both in the laboratory by experimental studies and in the field by patient collection of data and correlating them with the findings obtained in the laboratory. Experiments in biological control have, sometimes, failed when these studies have been neglected. *Bagrada cruciferarum* Kirk is a serious pest of cruciferous crops sporadically appearing to an epidemic form. Although mazie is a minor host of the pest, the parasite, *Hadrophanurus* species has been recorded only from the pest infesting this host plant. The conclusions derived from the population studies of the parasite with its effects on the bug population in maize can be utilised to achieve a measure of success in the control of the bug on cruciferous crops.

Some parasitised eggs of *B. cruciferarum* were observed on the leaves of maize during the kharif season of 1957 while the egg population of *Chilo zonellus* Swinhoe was being recorded to evaluate the effect of liberations of *Trichogramma evanescens minutum* Riley for the control of the borer. From these parasitised eggs of the bug, adults of *Hadrophanurus* species emerged out instead of *Trichogramma* (this is the first record of the genus from India). In 1958 while repeating the experiment on the borer control, special attention was, therefore, paid to count and collect the eggs of *B. cruciferarum* Kirk to confirm the occurrence of this parasite in nature and to evaluate its economic importance.

Material and Methods

The technique followed in the population studies of *Trichogramma* was utilised in the case of *Hadrophanurus* also. There were two blocks each consisting of six

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*Identified by the second author.
**Maize is a minor host of the pest as rearing of the bugs on it shows a very high mortality and only some nymphs reach the adult stage.

plots (half an acre each). The experiment covered an area of six acres. The distance between the two blocks was about half a mile. Each plot was separated from the other by a distance of 105 ft. In each plot there were 42 rows and each row was having about 140 plants. Thus there were about 5880 (140×42) plants per plot. In each observation five rows out of 42 rows were selected at random for taking observations. From each of these five rows 12 plants were cut from the soil surface. These 12 plants constituted every tenth plant of the row. In this way there were 60 plants per plot totalling 720 plants in all to be examined at every observation. There were eight such observations. Each of these 720 plants was examined thoroughly for the egg masses of *Bagrada*. The number of eggs on these plants was recorded (there were no eggs in the soil or debris in the field).

EXPERIMENTAL FINDINGS AND GENERAL OBSERVATIONS

B. cruciferarum Kirk lays its eggs on maize leaves mostly in batches of single layers. All the eggs are stuck together with their micropyle facing upwards. Large egg masses, sometimes, have (though rarely) two layers one upon another. The bottom layer generally consists of more than two third of the eggs while the top layer has less than one third of the eggs of the mass. Few scattered eggs are also encountered. All the scattered eggs and the egg masses consisting of eggs in single layer are parasitised comparatively more efficiently but the egg masses consisting of two layers decrease the coefficient of efficacy of the parasite as the eggs of the bottom layer are mostly inaccessible to the parasite. The number of eggs found during the course of the observations has been given in Table I.

TABLE I

Showing the number of Bagrada eggs parasitised by Hadrophanurus *sp.*

Number of eggs collected from

Treatment-plots							Control-plots						
**Replications Dates of observations	1	2	3	4	5	6	**Replications Dates of observations	1	2	3	4	5	6	Total
2– 9–58	..	—	..	...	—	—	3– 9–58	..	—	—	—	—	—	—
9– 9–58	10	—	54*	..	—	..	10– 9–58	—	25*	—	..	—	89	178
16– 9–58	8*	17*	—	—	—	44	17– 9–58	..	—	—	36*	—	4	109
23– 9–58	—	13	46*	28	—	—	24– 9–58	65	39	18	—	96 (86*+10)	—	305
30– 9–58	..	—	5	—	80*	—	1–10–58	—	9	—	—	—	—	94
7–10–58	—	—	—	—	—	—	8–10–58	—	—	—	—	—	—	—
14–10–58	—	—	—	—	—	—	15–10–58	—	—	—	—	—	—	—
23–10–58	—	—	—	—	—	—	24–10–58	..	—	—	—	—	—	—
Total	18	30	105	28	80	44		65	73	18	36	96	93	686

**These replications were meant mainly for evaluating the role of *Trichogramma* against *Chilo*.

*These eggs of *Bagrada cruciferarum* Kirk were parasitised by *Hadrophanurus* species.

The endoparasite, *Hadrophanurus* species distributes its progency singly in each of the host eggs. All parasitised eggs turn black (Text-fig. 1, Fig. 4) and a single active adult parasite emerges out from each host egg. The examination of 456 parasitised eggs (Table II) revealed no case of superparasitism. The percentage of parasitism reached as high as 85.1 per cent during the season. From the data it is obvious that the viability of eggs is 100 per cent under field conditions. In other words there has been no case in which parasitised eggs have not given rise to an adult wasp.
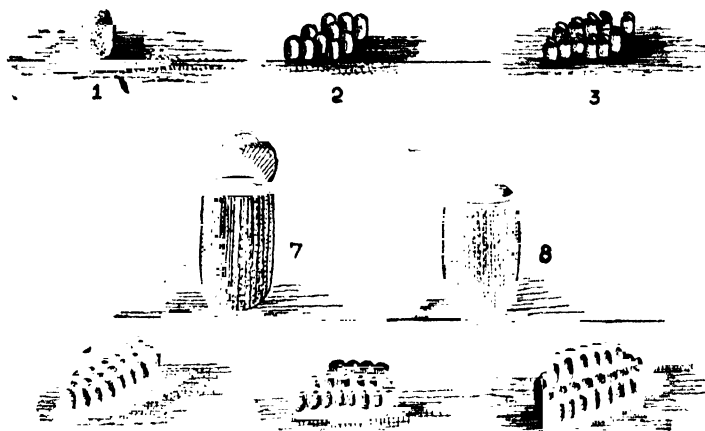
TEXT–FIG 1.

1. An egg of *Bagrada cruciferarum* Kirk .. (magnified)
2. An egg mass of *B. cruciferarum* Kirk .. „
3. Egg shells after the emergence of the nymphs .. „
4. Parasitised egg mass of *B. cruciferarum* Kirk .. „
5. Egg shells after the emergence of the parasites .. „
6. Parasitised egg mass having double layers .. „
7. Empty egg shell showing the emergence of the nymph „
8. Empty egg shell showing the emergence of the parasite „

TABLE II

Showing the percentage of parasitism by and the viability of Hadrophanurus *sp. as found during weekly observations*

| Dates of observations	Total no. of *Bagrada* eggs collected*	Number of *Bagrada* eggs parasitised	Percentage of parasitism	Numbr of parasites emerged*
2/ 3– 9–1958	—	—	—	—
9/10– 9–1958	178	79	44.4	79
16/17– 9–1958	109	61	56.0	61
23/24– 9–1958	305	236	77.4	236
30/ 1–9/10–1958	94	80	85.1	80
7/ 8– 10–1958	—	—	—	—
14/15– 10–1958	—	—	—	—
23/24– 10–1958	—	—	—	—
Total	686	456		456

The figures in the columns marked with an asterix are the basis for the estimation of the population per acre (Table III) of adults of *Hadrophanurus* species and eggs of *Bagrada cruciferarum* Kirk respectively.

TABLE III

Showing the population per acre of adults of Hadrophanurus *species and eggs of* Bagrada cruciferarum *Kirk*

| Dates of observations	No. of plants examined	No. of *Bagrada* eggs collected	No. of *parasites* emerged	No. of *Bagrada* eggs/ plant	No. of *parasites* per plant	Appr. no. of plants per acre	Total no. of parasites /acre	Total no. of Bagrada eggs/ acre
2/ 3 – 9–58	720	...	..	...	...	11760	—	—
9/10 – 9–58	720	178	79	0.25	0.11	11760	1294	2940
16/17 – 9–58	720	109	61	0.15	0.08	11760	941	1764
23/24 – 9–58	720	305	236	0.42	0.33	11760	3881	4939
30/ 1 –9/10–58	720	94	80	0.13	0.11	11760	1294	1529
7/ 8 – 10–58	720	..	...	...	...	11760	...	...
14 15 – 10–58	720	..	...	—	...	11760	...	...
23/24 – 10–58	720	..	...	—	...	11760	...	—
Total	686	456					7410	11172

The adult parasites emerge generally in the early hours of the day and the copulation takes place immediately after emergence. On cloudy and rainy days the parasites are less active. Further details of life history and technique of mass multiplication necessary for the biological control projects are being studied and will be published in due course.

LAY OUT OF BIOLOGICAL CONTROL EXPERIMENT IN MAIZE
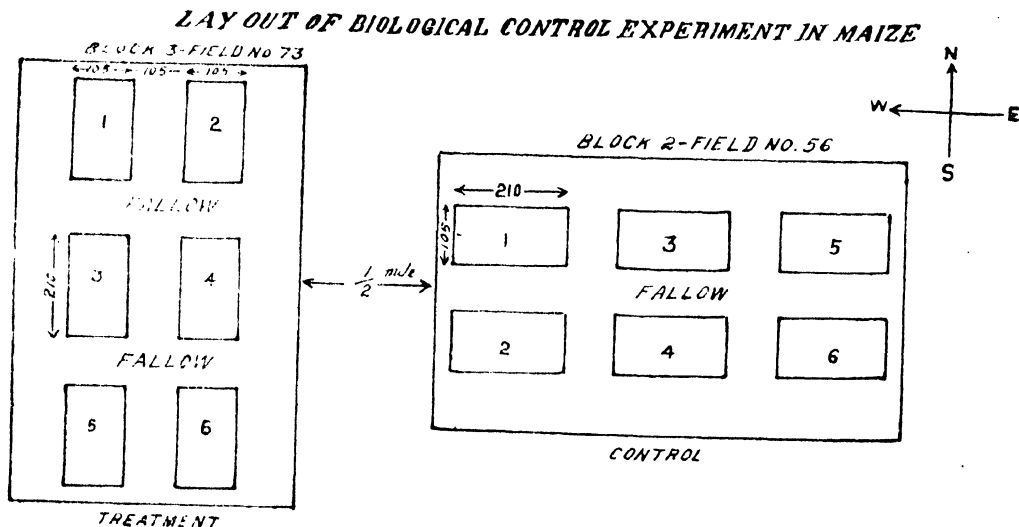
TEXT-FIG. 2.

DISCUSSION

Hutson (1935) reported that the eggs of *Bagrada* are laid on leaves, leaf-stalks and stems of cruciferous crops and Pruthi (1946) recorded "Contrary to the previous view, eggs were found to be laid in the soil and not on plants". The authors' observation is in support of the former worker at least in the case of maize as host plant. These altogether contradictory observations may be due to seasonal variations. During rainy season (the period of maize crop) when the soil is too wet and
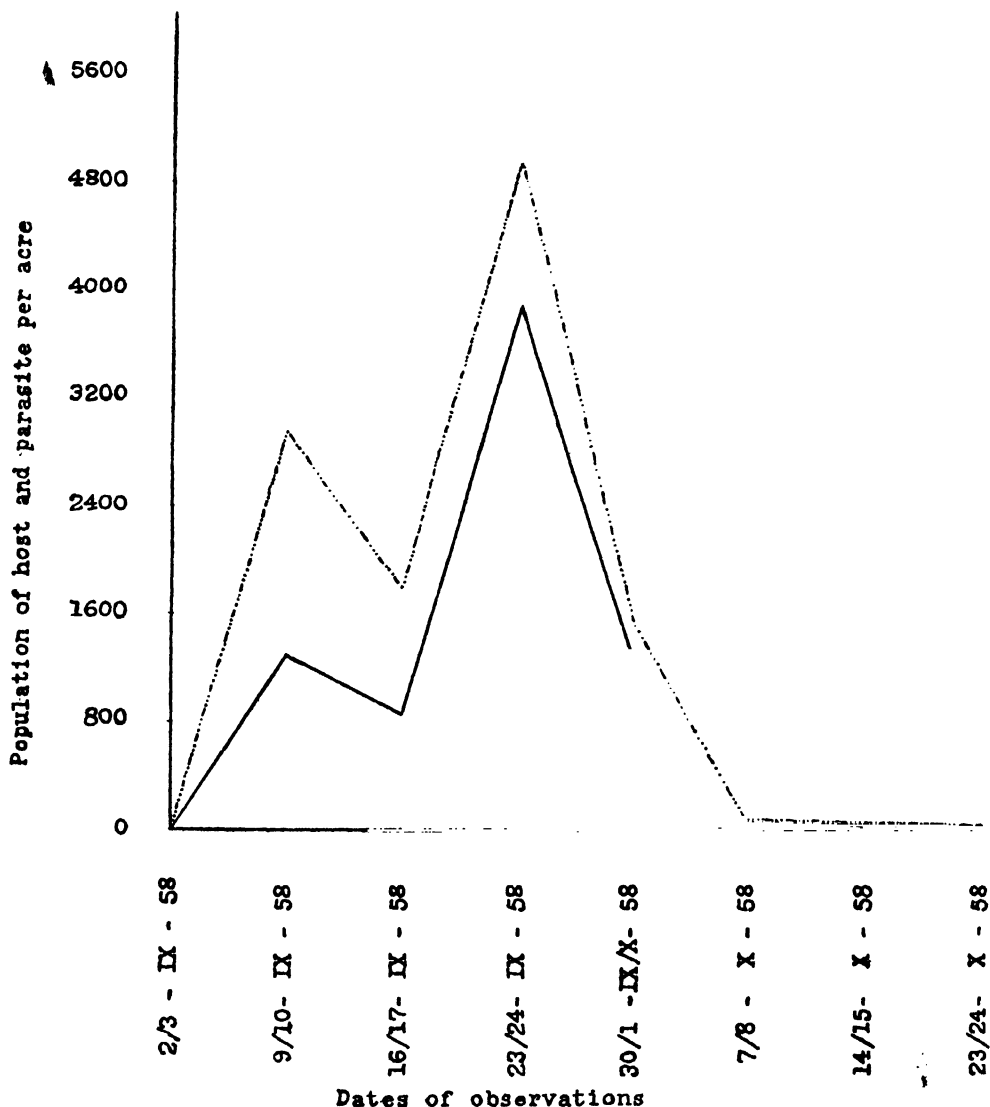
TEXT-FIG. 3.

Showing interaction of *Bagrada Cruciferarum Hadrophanurus* Kirk (..—..) and its parasite, *Hadrophanurus* sp. (———).

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the debris is almost in a rotting condition, the bug might have preferred plant surfaces for oviposition. In summer and severe cold weather when the atmosphere is too hot or cold and dry as the case may be, the bug might have chosen the debris and soil for the deposition of eggs. In both the cases the bug seeks both safety and better conditions for the development of its progeny. On leaf surfaces the eggs of the bug have been parasitised only by *Hadrophanurus* species and not by the two species of scelionids, viz. *Liophanurus samueli* Mani and *Tiphodytes* species parasitising the eggs in the soil as reported by Samuel (1944). But whether and to what extent the parasite is able to parasitise the eggs found in the soil (as in summer and winter season crops) is beyond the scope of the present investigations.

According to Isaac (1946) the population of insects per acre is more reliable than the population of insects per plant because in the latter both the number of plants as well as the number of insects per plant are variable, but in the former the acre is a fixed scale and only the population is variable. It is, therefore, that the weekly population per acre of adults of *Hadrophanurus* species and of the eggs of *Bagrada* have been calculated and given in Table III. The rate of increase of the population of the parasite is directly proportionate to the rate of increase of the population of the host. In other words when the host population was maximum, that is, 4839 eggs per acre in the last week of September, the population, of *Hadrophanurus* was also maximum, that is, 3381 parasites per acre in the same week. Similarly in other weeks the rise and fall in the population per acre of parasites were directly proportional to the rise and fall in the population per acre of the pest (Text-fig. 3). This intimate interaction and fluctuation between the host and parasite even at low population levels proves that the parasite is gifted with a strong searching capacity and greater tolerance to adverse conditions. This relationship may be more intensified in the cruciferous crops due to having lesser foliage than maize crop. Just because of this, it is possible that the parasite may prove to be a factor in the control of the pest attacking them.

From the data (Table III) it is obvious that the population of parasites increases to such an extent that it exterminates the host and ultimately proves fatal to the parasite itself. This is why in the last three observations there has been neither host nor parasite available in the field (Table I). There is another probability that host and parasite might have migrated to some other crop more preferable to them and which might have been the cause of their absence from the maize crop. This point needs survey of parasites in crops growing in the vicinity of maize crop in future.

REFERENCES

Hutson, J. C. (1935). The painted *Bagrada* bug, *Bagrada picta*. *Trop. Agriculturist*, **85**(3), 191–193.
Isaac, P. V. (1946). Report of the Imperial Entomologist. *Sci. Rep. Agric. Res. Inst., N. Delhi*, 1944-45, 73–79.
Pruthi, H. S. (1946). Report of the Imperial Entomologist. *Abridged Sci. Rep. Agric. Res. Inst., N. Delhi*, 1944–45, 64-71.
Samuel, C. K. (1944). Short notes and exhibits : Biological notes on two new egg-parasites of *Bagrada picta* Fabr. Pentatomidae. *Indian J. Ent.*, **4**(1), 92–93.

STUDIES ON THE AQUATIC INSECT FAUNA OF POONA (AQUATIC HETEROPTERA)

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(Communicated by B. S. Bhimachar, F.N.I.)

ABSTRACT

The paper gives results of the collection of aquatic Heteroptera made during last two years with brief notes on their habitats.

Over forty species belonging to eleven families are recorded and some are new records from this area.

Relative abundance of each group is determined and attempts are made to correlate the fluctuations with the climatic conditions.

Perhaps the most striking result of this study has been the demonstration that it is possible to define the habitats and climatic conditions which are favourable and characteristic to each group.

INTRODUCTION

The aquatic Heteroptera of Poona and adjoining areas have not been regularly studied in the past and no published account of their variety, seasonal abundance and their periodicity is available except a few notes of Annandale (1919) and Paiva (1919). The present paper gives results of my spare time collection of aquatic bugs with brief notes on their habitats. Regular collections are made at least four days in a week from various tanks, ponds, pools, streams, canals and the rivers. Moreover, a monthly record of chemical analysis of water collected from the same place, together with the meteorological data is maintained to correlate, at a later date, fluctuations in the populations of aquatic Heteroptera with the physical and chemical conditions of their environment.

Although some work has been carried out on the systematics of Heteroptera, our knowledge of the biology of the group is extremely limited (Miller 1956). Aquatic Heteroptera are often preyed upon by fish (e.g. trout) and examination of stomach contents of several fresh water fishes has revealed the remains of Gerridae, Corixidae and Pleidae. On the contrary, Belostomatidae and Notonectidae are voracious feeders and attack small fish, immature batrachians and molluscs. At a time when May-flies (Ephemeroptera) emerge, it has been observed that the trout are fatter and better flavoured (Miall 1903). Thus it is understandable that insect fauna of a given aquatic area exerts not only a potential but a real influence on the fishes living in it. For a pisciculturist, therefore, the knowledge of insect life of his fisheries is of as great importance as that of vegetation or oxygen content of these waters.

REVIEW OF PREVIOUS STUDIES

Distant (1903, 1906, 1910) is one of the earliest who contributed immensely to our knowledge of Hemipteran fauna and he deals with several genera of aquatic Heteroptera. Annandale (1919) pioneered the study of ecology of aquatic insects and his contributions have stimulated other workers in our country. Hora (1930, 1933) investigated the insect fauna of rapid streams with special reference to their adaptations in such an environment. Pruthi (1939) recorded some insects from

a hot spring in Kulu valley and also made a special investigation of insect fauna of certain highly saline ponds and tanks of the Punjab Salt Range.

Hutchinson (1933) found it necessary to revise some of the types of Notonectid and Corixid species described earlier. Hafiz and Mathai (1938) have listed some of the aquatic Hemiptera from Bihar. Hafiz and Pradhan (1947) also provide notes on the collection of aquatic Rhynchota from Orissa. Hafiz and Ribeiro (1939) have studied extensively the aquatic bugs of certain regions of Bihar State. Hutchinson (1940, 1944) has furnished a revision of Indian Corixidae. The Gerridae are dealt with by Brown (1949).

### Topographical and Meteorological Considerations

The two small rivers, the Mulla and the Mutha which are the tributaries of the great river Bhima run through the city of Poona and on whose confluence it is situated (Fig 1). It is in latitude 18° 31′ N and longitude 73° 51′ E. The altitude of the place is 1850 feet above the sea level and in straight line about 63 miles from
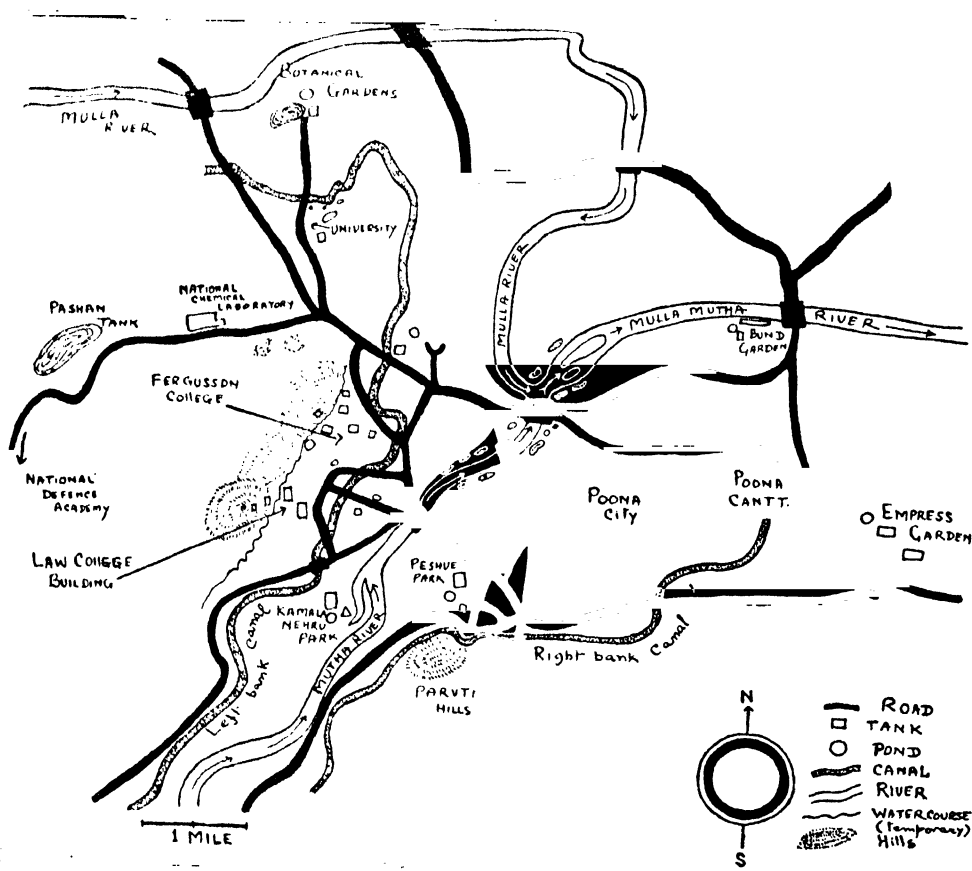
TEXT-FIG. 1.

Map of the part of Poona showing location of stations at which regular collections were made.

the west coast. The city is surrounded by uplands and hills. Owing to its position in the shadow behind the Western Ghats, the climate of Poona is dry most of the year. On account of its elevation and dryness, Poona is cool during nights even in summer In contrast to the maritime climate experienced by other near stations on the west coast, Poona enjoys a continental climate characterised by large diurnal ranges of temperature. Figs. 2 and 3 give normals of temperature and
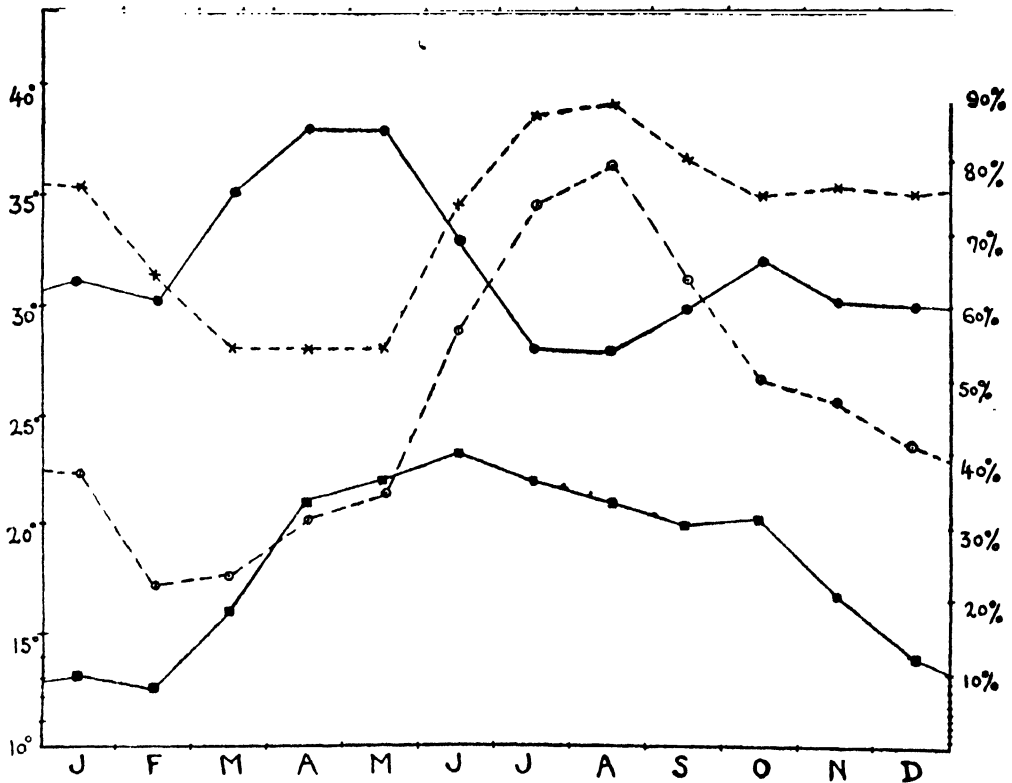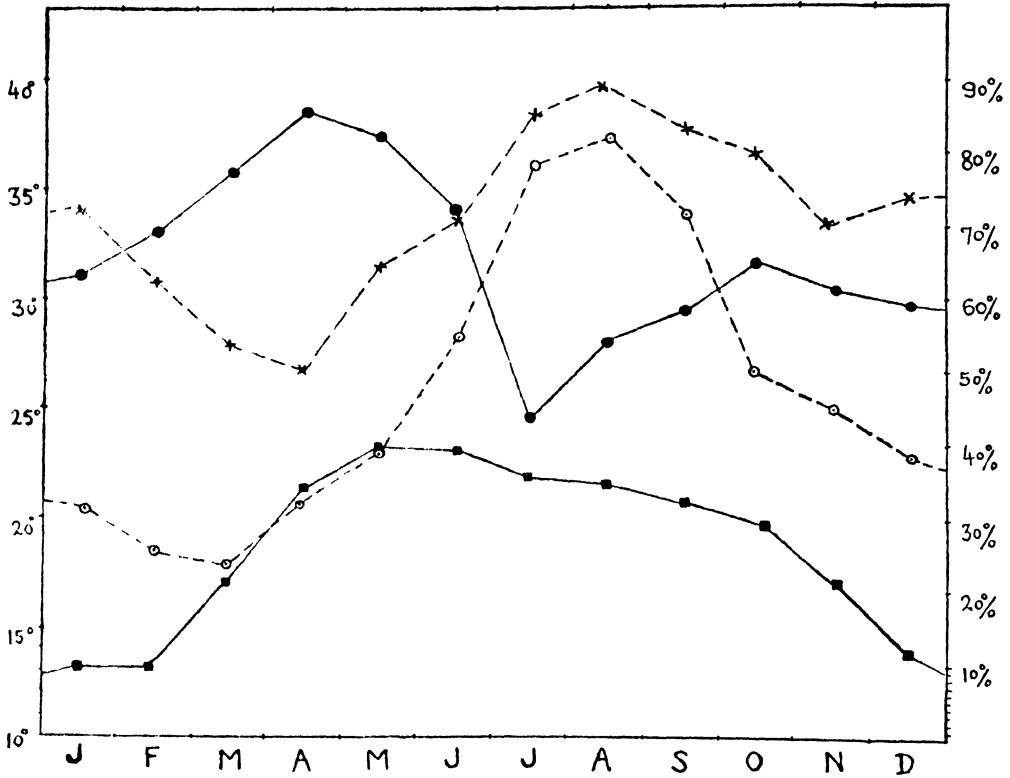

Poona : Monthly climatic means for 1957.
On left, temperature (Centigrade); monthly mean
maximum (●——●) and minimum (■——■)
On right, humidity %; at 0830 IST (× · · · · ×) and
at 1730 IST (⊙ · · · · · ⊙).

TEXT-FIG. 2.

humidity during the different months of the years 1957-58. Monthly average rainfall in mm. during the same period is given in Fig. 4. About 75 per cent of the annual rainfall in Poona occurs during the monsoon season, July being the wettest month. The thunderstorms usually preceed the hot season and render the air sultry. They are normally accompained by violent winds, sharp showers and hail. The year may be divided into three seasons, the cold season from November to February, the hot season from March to May and the wet season from June to October.

The work was carried out on the following lines : (i) To collect the specimens from different habitats listed below in different seasons, (ii) To note the characteristics of the habitat and some common insect associations and other incidental observations, (iii) To study the exact distribution and frequency of individual species, (iv) To study the seasonal changes in the composition of the insect fauna.

Poona : Monthly climate means for 1958.
On left, temperature (Centrigrade); monthly mean
    maximum (●———●) and minimum (■———■)
On right, humidity %; at 0830 IST (× - - - - ×) and
                at 1730 IST (⊙ - - - - - ⊙).

TEXT-FIG. 3.

Each site was usually visited at least once a week and the data collected and entered in a log-book. This collection includes practically all the species that were recorded by the early workers besides a fairly large number of species which may now be considered as new records from this area.

It may be mentioned here that each habitat was investigated by dredging with a net and explored as thoroughly as possible so that chances of defects in collecting are considerably reduced.

Monthly average rain-fall in mm. for 1957 ■

and 1958 ////
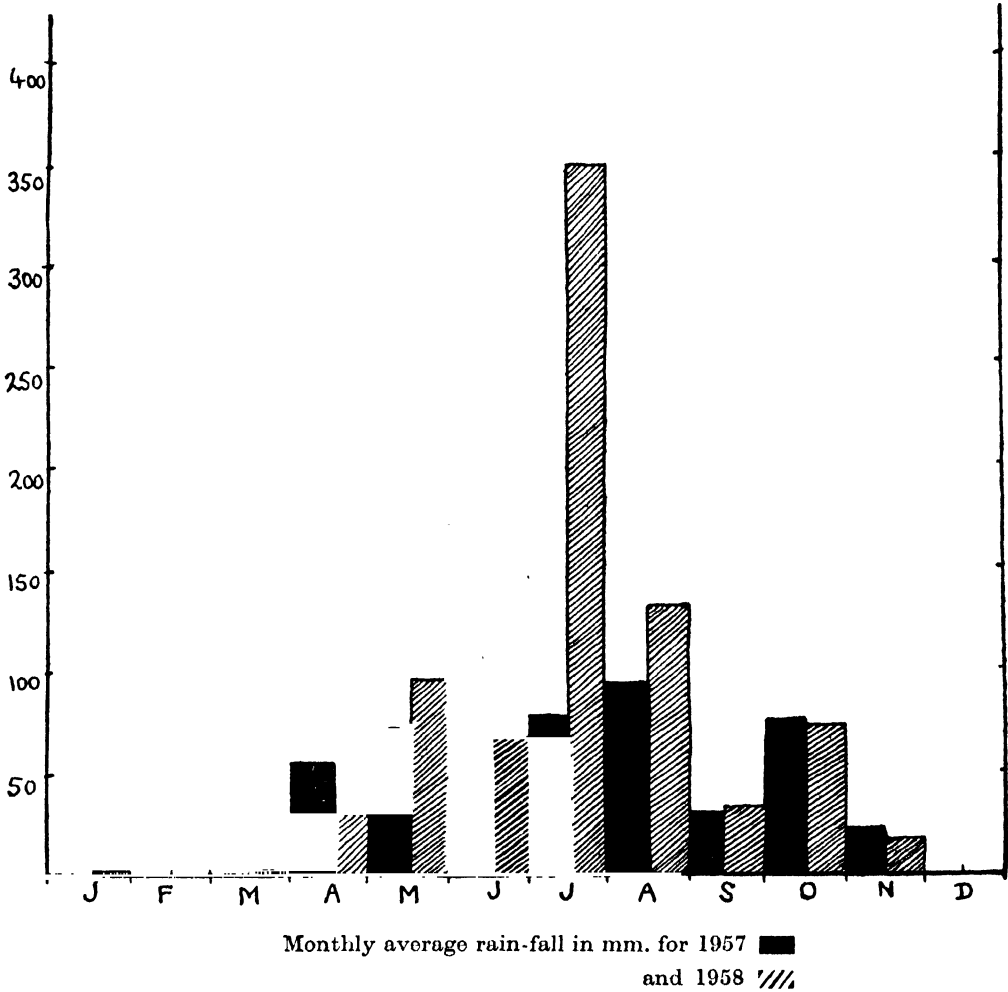
TEXT-FIG. 4.

LIST OF THE SPECIES AND HABITAT

The following table is the list of the species collected during the last two years. The types of habitat are given in an abbreviated form and the average of maximum and minimum temperatures, relative humidity and rainfall for the peak month are also provided.

ABBREVIATIONS

S—Stream;	P—Pond;	K—Tank;	R—River;
L—Pool;	C—Canal;	T—Temporary water course;	
O—Rocky;	M—Muddy;	V—Vegetation.	

TABLE I

| Species | Habitat | Average Max. & Min. Temp : R.H. and Rainfall for the peak months | |
		1957	1958
1	2	3	4
Cryptocerata (Hydrocorisae)			
I　Nepidae			
1　*Ranatra elongata* Fabr.	S,P,C,R,		
2　*R. filiformis* Fabr.	O,V.	28.2°C—21.8°C	24.5°C—22.2°C
3　*Leccotrephs maculatus* Fabr.		86%—74%	85%—78%
4　*L. ruber* (L.)		91.0 mm.	399.4 mm.
5　*L. grieus* Guér			
6　*L. elongatus* Mont.			
II　Belostomatidae			
7　*Sphaerodema molestum* Duf.	P,K,C,	30.3°C—20.2°C	29.9°C—14.4°C
8　*S. rusticum* F.	O,V.	80%—64%	83%—71%
		30.8 mm.	30.9 mm.
9　*Belostoma indicum* Lep. & Serv.	R,S,T,	32.6°C—12.8°C	32.3°C—20.2°C
10　*Belostoma* sp.	M,O,V.	75%—50%	80%—54%
		75.8 mm.	74.0 mm.
III　Naucoridae			
11　*Naucoris sordidus* Dist.	S,R,L,	28.1°C—21.3°C	28.3°C—22.0°C
12　*Heliocoris vicinus* Mont.	P,O,	87%—79%	89%—81%
13　*H. breviceps* Mont.	V.	91 mm.	134.5 mm.
14　*H. indicus* Spinn.			
IV　Corixidae			
15　*Corixa hieroglyphica* Duf.	D,C,T,	30.3°C—20.2°C	29.1°C—21.3°C
16　*C. lima* (Dixon)	R,L,S,	80%—64%	83%—71%
17　*Corixa* Sp.	M,(V)	30.3 mm.	30.9 mm.
18　*Micronecta minthe* Dist.			
V　Notonectidae			
19　*Notonecta glauca* Linn.	P,K,R,S,	32.6°C—12.8°C	32.3°C—20°C
20　*Enithares templetoni* Kirby	O,V.	75%—50%	80%—54%
21　*E. indica* Fabr.		75.8 mm.	74 mm.
22　*E. lactea* Paiva			
23　*Enithares* sp.			
24　*Anisops sardea* Schaff.	P,K,R,	30.3°C—20.2°C	29.1°C—21.3°C
25　*A. nivens* Fabr.	S,O,V.	80%—64%	83%—71%
26　*Anisops* sp.		30.8 mm.	30.9 mm.
VI　Pleidae			
27　*Plea pelopea* Dist.	C,T,S,	28.2°C—21.3°C	24.5°C—22.4°C
	R,V,M.	86%—74%	85%—78%
		77.5 mm.	399.4 mm.

TABLE I—*Contd.*

Species	Habitat	Average Max. & Min. Temp : R.H. and Rainfall for the peak months	
		1957	1958
1	2	3	4
Gymnocerata (Amphibiocorisae)			
VII Gerridae			
28 *Gerris fluviorum* F.	P,K,R,	32.6°C—12.8°C	32.3°C—20.0°C
29 *G. armata* Spin.	C,S,R,	75%—50%	80%—54%
30 *G. spinolae* L. & S.	O,V.	75.8 mm.	74.0 mm.
31 *Gerris* sp.			
32 *Metrocoris stali* Dohrn.			
33 *Onychotrechus rhexenor* Kirk.			
34 *Ptilomera laticaudata* Harw.			
VIII Hydrometridae			
35 *Hydrometra vittata* Stål.	S,K,C, O,V	28.2°C—21.3°C	24.5°C—22.4°C
36 *Lemnometra fluviorum* Fabr.	(Algae)	86%—74%	85%—78%
37 *Hydrometra* sp.		77.5 mm.	399.4 mm.
IX Hebridae			
38 *Hebrus bombayensis* Paiva	P,L,S,R, V (Mossy)	38.2°C—21.0°C	28.5°C—21.7°C
39 *Hebrus* sp.		54%—23%	50%—31%
		53.6 mm.	33.0 mm.
X Veliidae			
40 *Rhagovelia nigricans* (?) Burn.	S,C,T,R, O,V (Algae)	28.1°C—21.3°C	28.3°C—22.0°C
		87%—79%	89%—81%
		91.0 mm.	134.5 mm.
XI Pelogonidae			
41 *Ochterus marginatus*	S,R,C, O,V.	30.3°C—20.2°C	29.1°C—21.3°C
		80%—64%	83%—71%
		30.8 mm.	30.9 mm.

BIOLOGICAL OBSERVATIONS

Nepidae : The species belonging to this family were collected from streams, ponds, permanent canals and shallow parts of the river. Both, *Ranatra* and *Leccotrephes* were found climbing and crawling on the various aquatic plants. The former were also found inhabiting the bottom and walls of the large tanks. The ova, characterised by their long respiratory processes, were collected from the stems of aquatic plants in which they were embedded. Both the genera showed their characteristic peak in July and August. They prey on insects, crustaceans such as Daphnids etc. and other Arthropods. The phenomenon of catalepsy i.e. assuming a strange and rigid posture when disturbed or captured, has been observed in field as well as in the laboratory.

Belostomatidae : Some of the largest Heteroptera belong to this family. The two genera differ to some extent in their habitats. Both prefer waters with

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abundant vegetation. The two species of *Sphaerodema* were regularly collected from ponds and tanks whereas *Belostoma* were collected from rivers and the permanent canals indicating that the latter prefer slow-flowing waters. The representatives of this family showed their characteristic peak in September and October. *Belostoma* often fly in the nights from one canal or river to another and during flight were attracted to street lamps. Several specimens were collected near the mercury-vapour street lights. In habits they are very voracious, feeding upon small fishes, immature batrachians and other insects.

Naucoridae : The species belonging to this group were found in streams, pools and ponds all with rocky substratum. They seem to prefer waters with abundant emergent herbaceous vegetation among which they were seen to move and come to the surface periodically to replenish their air supply. Often they were found to congregate among the aquatic plants. The representatives of this group show characteristic peak in August and September. They are mostly predacious insects attacking other small arthropods.

Corixidae : This group is perhaps more interesting than the other and one of the chief characteristics of their habitat is the muddy substratum. They were present in very large numbers in temporary ponds and canals where the water was turbid and muddy. Their number was maximum during the rainy season particularly so during August and September. Occasionally they were found in loose shoals. They are mostly bottom dwellers and are phytophagous, feeding on the organic ooze, algae, diatoms and other vegetable matter. Some of the species, as shown in the above list, prefer waters in which there is a flourishing vegetation. Breeding seems to take place even in a most temporary pool or canal. It is now a common knowledge that in certain parts of Mexico the eggs of certain Corixids are used as food. Incidentally the eggs also make good food for poultry and fishes.

Notonectidae : The species belonging to this family were collected from slow flowing canals, ponds, tanks and rivers. Usually they prefer relatively still waters. The species of *Enithares* were collected from places where vegetation was ample. The remaining species of *Anisops* were collected from similar localities but seem to prefer cleaner and fresh waters. The representatives of *Notonecta* show two peaks, one in summer (May) and the other in rainy season. The first peak is due to the large number of neanides captured in their various developmental stages. In their feeding habits they are rapacious, attacking fry of fishes, young batrachians and crustacea. No other creatures should be confined with them as size is no deterent and they can negotiate with almost any prey. Eggs are glued to aquatic plants and other floating objects.

Pleidae : Some authors have included this group under Notonectidae. The main habitats of pleidae are still waters of ponds, canals and river where they live among aquatic vegetation. They prefer clear and fresh waters. They were always found in groups of ten or fifteen. Often they were seen clinging to floating vegetation. The group shows characteristic peak in August and September.

The Gymnocerata are much more interesting than the Cryptocerata. Many of them are transitional forms and fluctuation in their number seems to depend largely on rains irrespective of the seasons. This is particularly true of Gerridae. As can be seen from Figs. 4, 5 and 6, several scattered and occasional pre-monsoon and post-monsoon rains brought a large number of Gerridae with wings. This probably indicates that they were on their way to some permanent place and advantage was taken of these showers for their migration to fresh sites.

Gerridae : The various species of this group were collected from small streams, pools and canals covered with *Lemna*. Mostly they occurred singly or in loose shoals. During the latter half of the rainy season several pairs were found *in Copula*. Large number of eggs were collected from variety of aquatic plants (Tonapi, 1958). Gerridae mainly feed on dead and drowning insects.

Hydrometridae : The principal habitats of this group are margins of ponds, tanks and canals. The Hydrometridae frequent only calm waters covered by a thick layer of *Sphagnum*. They appear to subsist on dead prey. A few brachypterous specimens were also collected. They showed their characteristic peak during July and August.

Hebridae : The species representing Hebridae were usually collected during summer and especially when pre-monsoon rains visit this region. They occur mostly in loose shoals. When disturbed the specimens disperse only to assemble again. They prefer shady cool places and were collected from a variety of habitats, but always with abundant vegetation such as *Sphagnum*, *Lemna* etc. Occasionally Hebridae have also been collected from similar wet localities.

Veliidae : The only species representing this group was usually collected from slow flowing shallow canals. The habitat was devoid of any emergent herbacious vegetation. But the areas were covered with moss. The specimens were always in loose shoals of 20 to 30 individuals. Several specimens were found *in Copula* in field. The group shows its characteristic peak in August and September.

Pelogonidae : The only species of Pelogonous was collected from the banks of small canals and margins of ponds and tanks. On several occasions the exuvae of the neanides were seen floating in the fresh and clear water. They are active, predacious and semi-aquatic. Their peak period of relative abundance seems to be July, August and September.
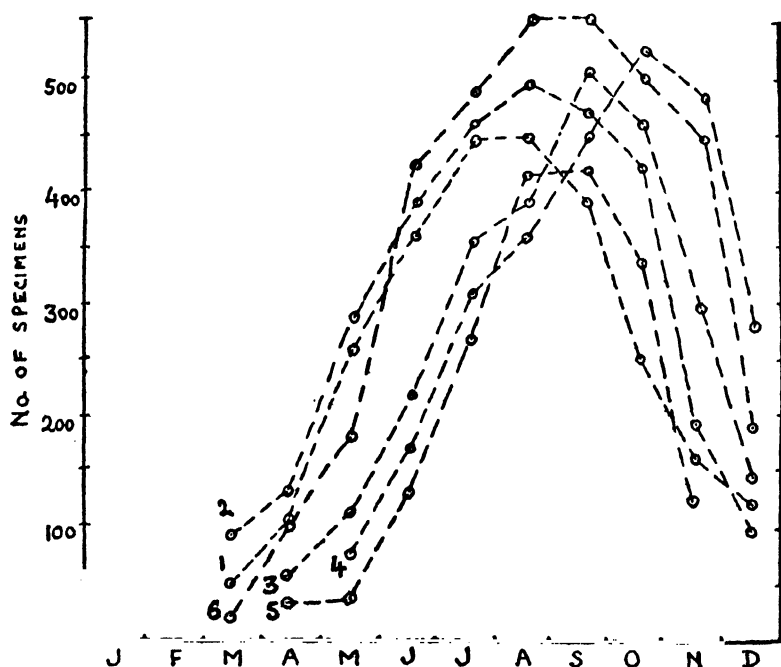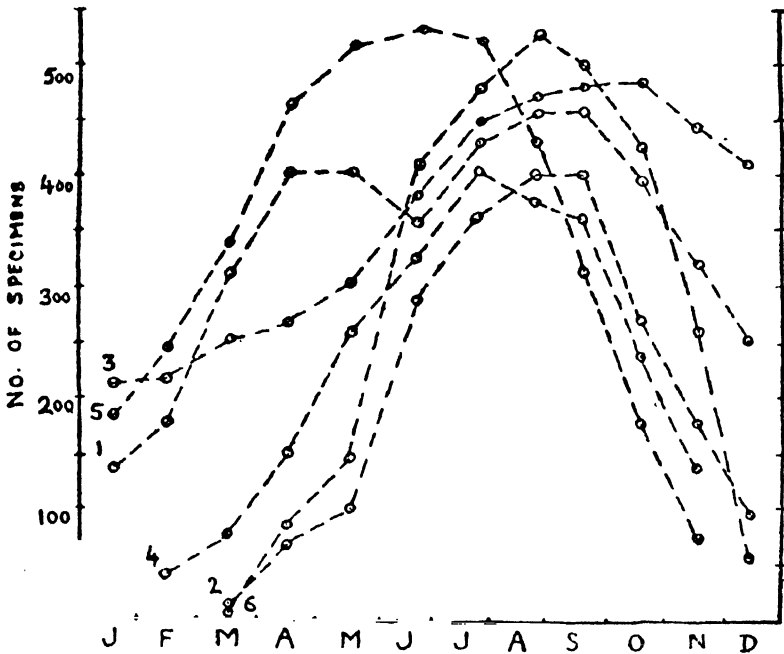

TEXT-FIG. 5.

Relative abundance and peak periods of different groups of aquatic Heteroptera.

1) *Ranatra elongata* F.	2) *Leccotrephes maculatus* F.
3) *Sphaerodema molestum* Duf.	4) *Belostoma* sp.
5) *Naucoris sordidus* Dist.	6) *Corixa hieroglyphica* Duf.

The present paper is mainly of a qualitative nature and aims at giving some nformation on the relative abundance of some groups of Heteroptera during the

different months of the year. For the sake of brevity, details of the daily reports, number of specimens collected and/or counted and the type of habitat in which each species occurs are not included in this paper. It is always difficult to evolve a suitable estimation technique to indicate the fluctuation in the insect population due to seasonal differences alone. Moreover, errors in sampling procedures add to the difficulties. However, attempt has been made to establish the "normal abundance" of each species by using an average count of that species for a given period and a given quantity of water. Fluctuations above and below this "Norm" are considered to be indicative of actual changes in the population. Not all species recorded here have been subjected to such analysis. A representative of each family, collection of which is particularly thorough, has been selected and fluctuation in their number is shown in Figs. 5 and 6.

TEXT-FIG. 6.

Relative abundance and peak periods of different groups of aquatic Heteroptera.
1) *Enithares indica* F. 2) *Plea pelopea* Dist.
3) *Gerris fluviorum* F. 4) *Hydrometra* sp.
5) *Hebrus bombanyansis* Paiva. 6) *Rhagovelia nigricans* Burn.

Following table (number 2) gives the list of the families and the peak months of their incidence.

TABLE II

Family	Month
Nepidae	
Ranatra	July
Leccotrephes	August
Belostomatidae	
Sphaerodema	September
Belostoma	October
Naucoridae	
Naucoris	August
Heliocoris	September
Corixidae	
Corixa	August
Micronecta	September
Notonectidae	
Enithares	October
Anisops	September
(Neanides)	April
Pleidae	
Plea	August/September
Gerridae	
Gerris	October
Onychotrechus	September
Hydrometridae	
Hydrometra	July
Hebridae	
Hebrus	April
Veliidae	
Rhagovelia	August

So far as the region under consideration is concerned, it may be inferred from the above information that the various groups of aquatic Heteroptera require specific climatic conditions during which they show their peak number. Thus, Nepidae require below average temperatures, narrow range of relative humidity and average rainfall. Belostomatidae prefer above average temperatures, a very wide range of relative humidity and below average rainfall. Naucoridae require below average temperature, narrow range of relative humidity and above average rainfall. Corixidae also require below average temperatures but a wide range of relative humidity and average rainfall. Notonectidae require above average temperatures, wide range of relative humidity and average rainfall. Anisopinae, although belonging to the same group, show preference to lower temperatures. Pleidae show their peak during periods when temperatures are below average, range of relative humidity is very narrow and rainfall above average. Gerridae show their peak when the temperatures are above average, relative humidity has a wide range and rainfall is average. Hydrometridae require below average temperature, narrow range of relative humidity and above average rainfall. On the other hand Hebridae are abundant when the temperatures are above average, range of relative humidity is wide and rainfall below average. Veliidae show their peak when temperatures are average, range of relative humidity is narrow and rainfall average. Pelogonidae also require average temperature, narrow range of relative humidity and average rainfall.*

It may be inferred from Figs. 5, 6 and Tables 1 and 2 together with the data in the log-book that the biological year can roughly be divided into four periods

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*Bi-annual average of temperature $\pm 10°C$, of R.H. $\pm 30\%$ and of rainfall ± 30 mm.

according to variation in collection. The rainy season i.e. July to September seems to be the most favourable months of the year for Amphibiocorisae as well as Hydrocorisae and a large number of these groups appear at frequent intervals. From October there is a decrease in their number and variety as only transitional forms occur in fluctuating numbers. And during winter months i.e. December, January and February the collection is both poor in quality as well as quantity. However, during summer i.e. March, April and May although there is an appreciable decrease in their number several groups such as Hebridae (Amphibiocorisae) and—Notonectidae (Hydrocorisae) are available in ascending numbers.

Not withstanding these divisions, any favourable change in the climatic conditions brings suddenly a large number of variety of Heteroptera. As can be seen from the rainfall data of 1958 compared with that of 1957 during relatively colder months. viz. January, February and March. scattered and occasional rains brought a large number of Heteroptera where there were none. A list of the number of species collected during that period shows that factors such as rainfall, relative humidity and temperature, etc., have an augmentative effect on the fluctuations in the Heteropteran population. It is hoped that continuation of this study for longer period in duration and intensity will shed further light on the factors that affect the Heteropteran population.

REFERENCES

Annandale, N. (1919). The fauna of certain streams in the Bombay Presidency, *Rec. Indian Mus.* **16**, 109–161.

Brown, E. S. (1949). A new species of *Gerris. Ann. Mag. nat. Hist.*, **1**, 892–912.

———— (1951). The relation between migration and type of habitat in aquatic insects, with special reference to certain species of *Corixidae. Proc. zool. Soc. Lond.*, **121**, 539–45.

Butler, P. M. and Popham, E. J. (1958). The effect of floods of 1953 on the aquatic insect fauna of spurn (Yorkshire). *Proc. R. ent. Soc. Lond.*, A, **33**, 149–158.

Distant, W. L. (1903). The Fauna of British India including Ceylon and Burma (Rhynchota)-**2**.

———— (1906). *Ibid.*, **3**.

———— (1910). *Ibid.*, **5**.

Hafiz, H. A. and Mathai, G. (1938). On the collection of aquatic Rhynchota from the Rajmahal Hills, Bihar. *Rec. Indian Mus.*, **40**, 207–210.

Hafiz, H. A. and Pradhan, K. S. (1947). Notes on a collection of aquatic Rhynchota from the Patna State, Orissa, with a description of two new species. *Ibid.*, **45**, 347–376.

Hafiz, H. A. and Ribeiro, S. (1939). On a further collection of aquatic Rhynchota from the Rajmahal Hills, Bihar, with a description of *Micronecta scutala* sp. Nov. *Ibid.*, **43**, 73–85.

Hora, S. L. (1927). Animal life in torrential streams. *J. Bombay nat. Hist. Soc.*, **32**, 116–126.

———— (1930). Ecology, Bionomics and Evolutions of torrential fauna, *Phil. Trans.* **218**,171.

———— (1933). Silken shelters of torrential insect larvae. *Curr. Sci.*, **1**, 341–42.

Hutchinson, G. E. (1940). A revision of Corixidae of India and adjacent regions. *Trans. Conn. Acad. Arts Sci.*, **33**, 339,476.

———— (1944). Notes on Hemiptera. *Ann. Mag. nat. Hist. (II)*, **11**, 769–778.

Hynes, H. B. N. (1955). Biological notes on some East African aquatic Heteroptera. *Proc. R. ent. Soc. Lond.*, (A), **30**, 43–54.

Miall, L. C. (1903). The natural history of aquatic insects. MacMillan & Co., London.

Miller, N. C. E. (1956). The Biology of Heteroptera, Leonard Hill Ltd., London.

Paiva, C. A. (1919). Aquatic and semi-aquatic Rhynchota from Satara and Poona districts. *Rec. Indian Mus.*, **16**, 152–158.

Pruthi, H. S. (1939). Record of some insects from a hot spring in Kulu valley, Punjab. *Indian J. Ent.*, **1**, 65–67.

Tonapi, G. T. (1959). A note on the eggs of *Gerris fluviorum* F. with a brief description of its neanide. *Ent. mon. Mag.* (In Press).

GERMINATION OF SPORES AND PROTHALLI IN TWO SPECIES OF *NEPHROLEPIS*, *N. EXALTATA* Schott. AND *N. ACUTA* Presl

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ABSTRACT

The paper gives an account of prothalli in two species of *Nephrolepis*, *N. exaltata* Schott. and *N. acuta* Presl obtained in nature and of those grown in cultures. These prothalli conform to hermaphrodite cordate type of prothallus common in many Davalloid ferns. They are 2-lobed but the lobes are not very broad or truncate. They form antheridiophores as in *Stenochlaena* and have hairs. They are easily propagated vegetatively by gemmae arising from any marginal cell of the prothallus. Scalariform tracheids were seen in the region of cushion in the prothalli of *N. exaltata* but not in those of *N. acuta*.

INTRODUCTION

The Davalloid genus *Nephrolepis* consists of thirty species, of which eight occur in India. Blatter and d'Almeida (1922) mention six species as occurring in the Bombay State, of which *N. exaltata* and its several varieties are often cultivated. These differ quite a lot in their genetic constitution (Conley, 1945). An account of sporogenesis in this species has been given by Mahabalé and Gorji (1951), and its anatomy has been described by Case (1932). The genus has attracted a number of workers*; but to the best of our knowledge, germination of spores and prothalli in many species of this genus are not known. The structure of adult prothallus in an unknown species of the genus has been given by Smith (1955), and recently an account of the germination of spores and prothalli in a facultatively epiphytic species, *N. paucifrondosa* d'Alm., has been given by Mahabalé and Javalgekar (1959). The present paper gives an account of prothalli in two common species of *Nephrolepis*, *N. exaltata* Schott. and *N. acuta* Presl which are quite common in Indian gardens.

MATERIAL AND METHODS

Some plants of *N. exaltata* were found growing wild in the crevices of rocks on a hill, called Raoli Hill, in Bombay Island at an altitude of about 200' above the sea level by one of us (T.S.M.) who also found there prothalli growing wild in December, 1948. These were collected and studied. Mature spores of the same species were collected from wild as well as cultivated plants in March, 1949, and were sown on culture media such as Knop's solution, potato-agar, etc. They germinated readily and formed prothalli in about 50 days.

Sporelings and mature prothalli were fixed in Navaschin's fluid, stained with Delafield's haematoxylin and mounted as a whole in Canada balsam (Pl. XIII, Fig. 2).

GERMINATION OF SPORES AND PROTHALLI

(A) *N. exaltata* Schott.—Mature spores in this species are spherical or semi-lunar, $70\text{-}75\mu \times 90\text{-}95\mu$. They are brown in colour, with thin endosporium and

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* For previous literature see Mahabalé and Javalgekar, (1959).

thick exosporium studded with small warts (Fig. 1). They become large by intake of water and show signs of germination after 60-68 hours (Figs. 2-4). Large, yellow oil-globules develop in them during this period and an opening is formed at the tri-radiate mark after about 4 days. The germ-tube comes out of it and many chloroplasts develop in it. They line the cell wall inside (Fig. 5). The first division is transverse separating a large, basal cell from an elongated upper cell. The first rhizoid is without chloroplasts, and generally comes out of the basal cell, the upper green cell giving rise to prothallial cells (Fig. 6). They, however, were seen to have been formed in other ways as shown in Figs. 9-10.

The prothallial cell divided transversely to form a 4-18-celled uniseriate filament (Figs. 7, 8 and 14). Branching is common at this stage (Fig. 9), all divisions so far in a young filament being transverse. A few longitudinal divisions, however, may be seen in the penultimate cell. Two oblique walls in the terminal cell establish a meristematic cell. As the divisions in meristem take place a spatula-shaped prothallus is formed in about 15 days (Fig. 15). Its further growth is rather slow and prothalli mature in 50 days.

An adult prothallus of this species is large, green, heart-shaped and 0.3 cm.× 0.5 cm. (Fig. 17 and Pl. XIII, Fig. 3). It has a large, raised cushion on the ventral side. It is 4-6-celled thick in the region of cushion and one-celled elsewhere. Its cells are polygonal and are lined by numerous chloroplasts. Filiform, unicellular, glandular hairs occur all over the prothallus (Figs. 11, 16-17 : h). The notch is deep and 4-7 meristematic cells lie in it (Fig. 17).

The prothallus is hermaphrodite in wild state ; but in culture material a few prothalli bearing only antheridia were always seen. Possibly there is protandry in the species. The antheridia-bearing prothalli are small, spatulate or irregular in shape and ameristic. In typical hermaphrodite prothallus antheridia appear first antero-posteriorly and lie on the posterior part of the cushion at maturity. They continue to be formed even after the archegonia have been formed and matured. A mature antheridium is large and spherical. It is raised on a one-celled stalk and consists of two ring cells, and a cap cell (Fig. 29). It has 32 spermatocytes. Spermatozoids have $1\frac{1}{2}$ coils each and they come out of the opening formed by the overthrow of cap cell. Their development was normal (Figs. 24-29).

Many archegonia are formed and lie on the anterior part of the cushion at maturity (Fig. 17). Archegonia are long and turned obliquely towards the posterior end. Their necks are 4-6-celled in each row (Figs. 17 and 23).

Abnormal prothalli.—Many abnormal prothalli were seen in this species, commonest amongst them being branched prothalli. In some of the branched prothalli marginal cells developed short, uniseriate branches, which produced an antheridium terminally, the whole structure resembling an antheridiophore (Figs. 11 and 12).

EXPLANATION OF TEXT-FIG. 1.

Figs. 1-29. *Nephrolepis exaltata* Schott. Germination of spores, prothalli and development of reproductive organs. Fig. 1. A spore × 210. Figs. 2-5. Early stages in the development of prothallus (Fig. 2 × 210; Figs. 3-4 × 165; Fig. 5 × 126). Figs. 6-10 and 14. Later stages in the development of prothallus (Fig. 6 × 165; Figs. 7-8 × 70; Figs. 9-10 × 126 ; Fig. 14 × 70) Fig. 11. A part of Fig. 12 magnified to show the structure of an antheridiophore : *ant*—antheridiophore; *h*—glandular hair × 165. Fig. 12. A prothallus with antheridiophores : *ant*—antheridiophores; *an*—antheridia × 8. Fig. 13. An abnormal prothallus showing tracheids in the cushion : *tr*—tracheids × 8. Fig. 15. A spatulate prothallus × 70. Fig. 16. A glandular hair—*h* magnified × 420. Fig. 17. A typical adult prothallus : *an*—antheridia; *ar*—archegonia; *h*—glandular hair × 70. Figs. 18-19. Parts of Fig. 13 magnified to show details of tracheids : *tr*—tracheids × 210. Figs. 20-23. Development of archegonium × 210. Figs. 24-29. Development of antheridium × 210.
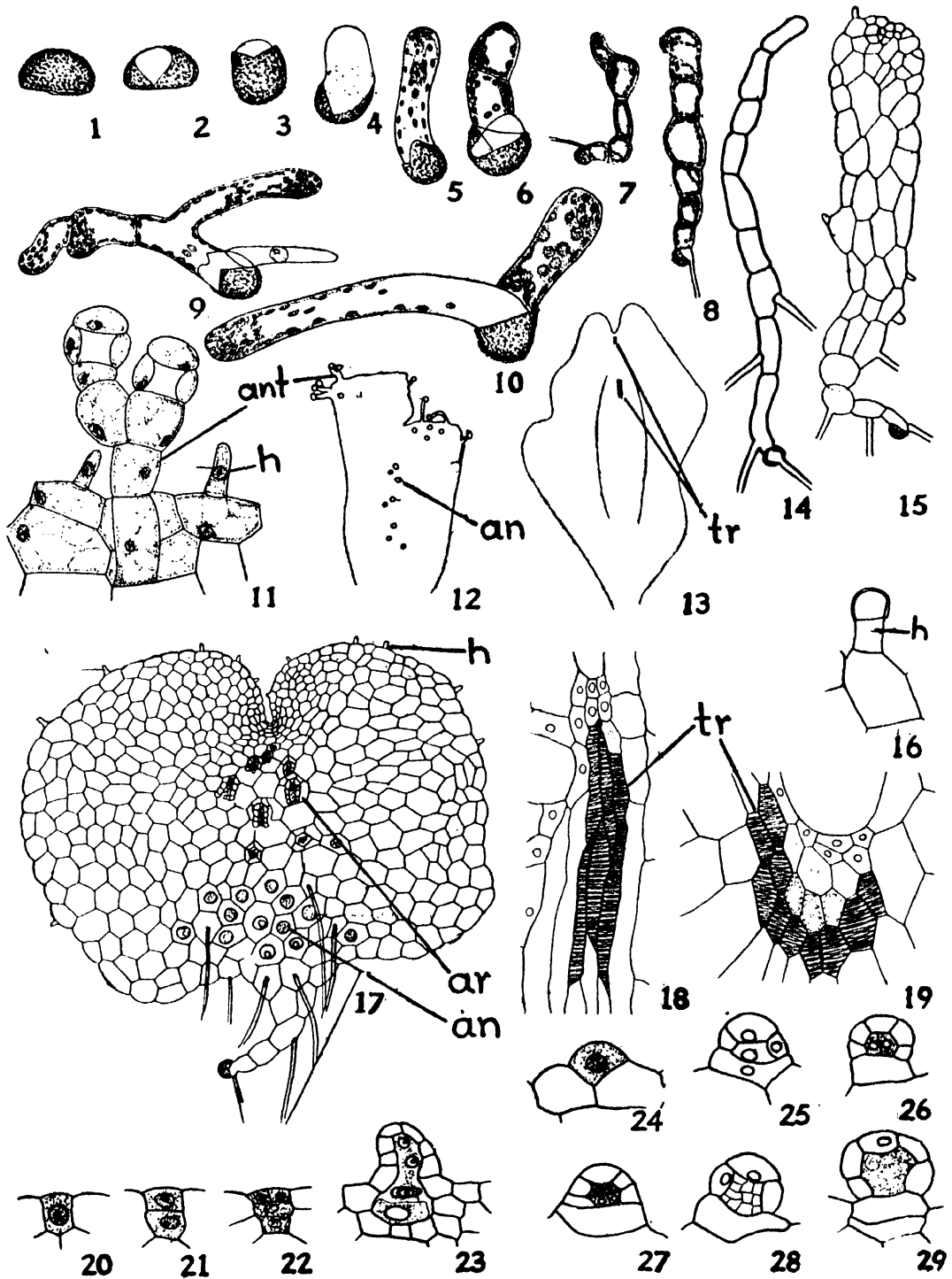
TEXT-FIG. 1.

In some of the older prothalli tracheids were seen to have been developed both in the anterior and posterior part of the cushion (Pl. XIII Fig. 1—*tr* and Text-Fig. 1 : 13, 18 and 19 : *tr*-tracheids).

Vegetative propagation and regeneration of older prothalli by the formation of new prothalli from any single cell of the margin of the prothallus was not uncommon. In such prothalli, any one of its cells functioned as a gemma and gave rise to prothallus and later developed reproductive organs. Prothalli with irregular shapes were quite common as shown in Pl. XIII, Fig. 2, upper row.

Development of reproductive organs.—Antheridia and archegonia develop as in other Polypodiaceous ferns (Figs. 20-29). The second ring cell in an antheridium, however, is sometimes suppressed, a mature antheridium having had only one ring cell (Figs. 11 and 28). A mature archegonium has long neck of 4-6 cells in each of the four rows, a binucleate neck canal cell, a venter canal cell and an egg (Fig. 23).

Sporophytes develop normally and have first two or three leaves simple : later ones are pinnati-partite. But in all cases their veins were dichotomising (Pl. XIII, Fig. 3 : *sp*-sporophyte).

PROTHALLI UNDER NATURAL CONDITIONS

The prothalli found wild under natural conditions at Raoli Hill are large, 0.7 cm. to 0.9 cm. long, dark green in colour, and hermaphrodite. They had a massive cushion 7–12 cells thick. Tracheids were found to have been developed in them also.
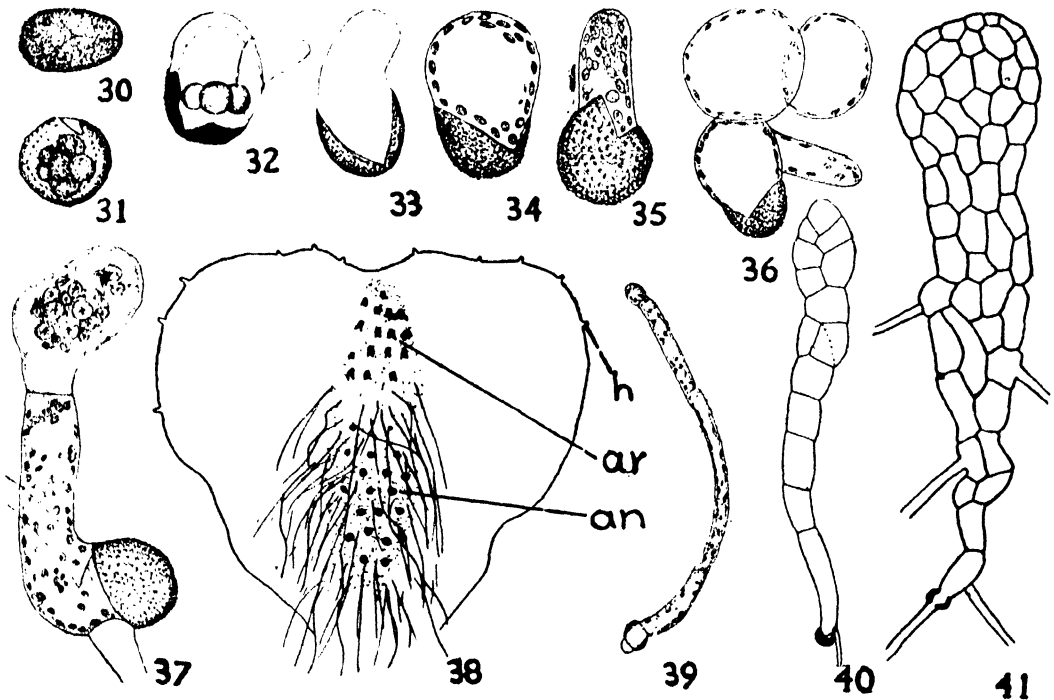
TEXT-FIG. 2.

FIGS. 30-41. *Nephrolepis acuta* Presl. Germination of spores and prothalli. Fig. 30. A spore ×210. Figs. 31-35. Early stages in the development of prothallus ×210. Figs. 36-37. Later stages in the development of prothallus (Fig. 36 ×126; Fig. 37 ×210) Fig. 38. A typical adult prothallus : *an*—antheridia; *ar*—archegonia; *h*—glandular hair ×38. Figs. 39-41. Later stages of developing prothalli ×70.

Fig. 1.   *Nephrolepis exaltata* Schott. A part of prothallial cushion with tracheids—*tr* × 420.
Fig. 2.   Abnormal prothalli of *Nephrolepis exaltata* Schott. (upper row) and *N. acuta* Presl (lower row) × 3.
Fig. 3.   *Nephrolepis exaltata* Schott. Adult prothalli: *sp*—sporophyte × 3.

Glandular hairs were also present in these prothalli similar to those on the prothalli grown in cultures, showing thereby, that they are characteristic of the gametophytes of *Nephrolepis*. No small male prothalli were found under natural conditions.

(B) *N. acuta* Presl—The spores in this species vary very much in size and shape, the largest among them being 120μ, almost double the size of the smallest, 70μ. The large ones are spherical and the smaller ones semilunar (Figs. 31 and 30). Size and shapes intermediate between these two were also found. Each spore has a dark brown body, small warts and a triradiate mark (Fig. 30).

The spores germinate slowly, develop oil globules in 76 hours, and the germ tubes came out of the pore at the triradiate mark (Figs. 30–35). The first division is transverse, relationship of the first rhizoid to the prothallial cell being the same as in *N. exaltata*. The second and subsequent divisions are transverse and form a 5-7-celled uniseriate filament in 20 days (Figs. 36, 37 and 39). Two oblique-walls in the terminal cell are cut off and form the growing point (Fig. 40). A spatulate prothallus is formed in 32 days (Fig. 41). It matured in 50–60 days.

Mature prothallus in this species is smaller than that in *N. exaltata*, being 0.4 cm. $\times 0.6$ cm. It is green, heart-shaped, with a shallow notch, and is covered by glandular hairs found all over its surface (Fig. 38). 4-6-celled thick cushion is small and has numerous rhizoids. Prothalli were mostly hermaphrodite (Fig. 38). But in this species male prothalli were found sometimes. Many antheridia are found on a prothallus. Archegonia are formed later. They are restricted to its anterior part. Antheridiophores occurred here also but were not very conspicuous in this species. A mature antheridium has two ring cells, a cap cell and 32 spermatocytes. Archegonia have longer necks, there being 7-8 cells in each of the four rows of the neck cells. No tracheids were noticed in the prothalli of this species ; but branching of filaments, vegetative propagation, regeneration by gemma-like cells, formation of antheridiophores, irregularly shaped prothalli were not infrequent (Pl. XIII, Fig. 2, lower row).

SUMMARY AND CONCLUSIONS

Germination of spores and prothalli were studied in two common species of *Nephrolepis*, *N. exaltata* Schott. and *N. acuta* Presl. Spores germinated readily and formed adult prothalli in 50 days in *N. exaltata* and in 70 days in *N. acuta*. Adult prothalli of *N. exaltata* are large, massive and deeply notched as compared to those in *N. acuta*. Branching of young, filamentous sporelings, vegetative propagation, regeneration by gemma-like bodies, formation of small male prothalli were common to both.

Antheridiophores were similar to those in *Stenochlāena* described by Stokey (1951) and were seen in both the species. Tracheids were noticed in the anterior and posterior part of the cushion in some prothalli of *N. exaltata* (Pl. XIII, Fig. 1), but not in *N. acuta*. Similar tracheids have been reported in the prothalli of *Aneimia*, *Adiantum* and *Lomaria*. Development of reproductive organs and young sporophytes appeared to be normal. In general it may be said that the prothalli in these two species conform to harmaphrodite, cordate type of prothallus, noticeable in many other Davalloid ferns. The lobes here, however, are not so broad as those in the prothallus of an unknown species illustrated by Smith (1955, p. 353). Formation of antheridiophores, gemma-like cells, tracheids in the prothalli in the region of cushion are novel features of this genus which have not been previously recorded.

REFERENCES

BLATTER, E. and D'ALMEIDA, J. F. R. (1922). The Ferns of Bombay.

CASE, I. M. (1932). The development of the sorus in some species of *Nephrolepis*. *Trans. Roy. Soc. Edinb.*, **57**, 259-276.

CONLEY, M. A. (1945). An anatomical and cytological study of *Nephrolepis exaltata* and some of its varieties. *Ohio Univ. Absts.* [Ex. *Biol. Abstr.*, **20**, (8), p. 1568, October, 1946].

MAHABALÉ, T. S. and GORJI, G. H. (1951). Sporogenesis in *Nephrolepis exaltata* Schott., *Bombay Univ. J.*, **20** (3), 66-77.

MAHABALÉ, T. S. and JAVALGEKAR, S. R. (1959). On a tuberiferous species of *Nephrolepis*, *N. paucifrondosa* d'Alm. *Ibid.*, **27** (5), 21-27.

STOKEY, ALMA G. (1951). The contribution by the gametophyte to the classification of the homosporous ferns. *Phytomorph.*, **1**, 39-58.

SMITH, G. M. (1955). Cryptogamic Botany. Vol. II, 2nd Ed., p. 353.

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